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### *ERRATA*

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE, VOL. 12, PART I

Page 128, line 34, *for* 'cellulosedc-' *read* 'cellulose.de-'

Page 151, heading of Table X, *for* 'electrodialys is' *read* 'electrodialysis'

Page 166, Table I, Col. 7, sub-head, *for* 'Acres'  
Gunthas *read* 'Acres Gunthas'



# ORIGINAL ARTICLES

## STUDIES IN VERNALIZATION OF MUSTARD (*BRASSICA JUNCEA*)

BY

B. SEN

AND

S. C. CHAKRAVARTI

*Vivekananda Laboratory, Almora, United Provinces*

(Received for publication on 15 September 1941)

(With Plates I and II and three text-figures)

### INTRODUCTION

VERNALIZATION experiments are based on Lysenko's theory of phasic development of annual seed crops. A comprehensive summary of the theory and of the experimental work on vernalization will be found in Bulletin 17 [1935] of the Imperial Bureau of Plant Genetics. The chief concepts involved in Lysenko's theory are : (i) growth of a plant is associated with increase in size and weight, and development with a sequence of qualitative internal changes without which a plant cannot reach its reproductive stage ; the rates of growth and of development are independent of each other, and environmental factors favourable for growth (vegetative cycle) may or may not be favourable for the development processes (reproductive cycle) ; (ii) there are several distinct steps (phases) in the developmental cycle, each with its specific environmental requirements and each depending for its initiation on the completion of the preceding stage, and if the requirements of any of the phases be lacking in the environment, the plant will develop as far as that phase and no further ; (iii) as soon as growth of the embryo of a seed starts, it can be treated from a physiological point of view as a growing plant. In the light of experimental evidence some of these concepts now have to be modified. Whyte [1939] aptly points out ' that the development, at least of the first two phases, can be maintained only by a certain minimum of growth and that non-growing seeds cannot be vernalized ' . The present knowledge of true relationship between growth and development is still too meagre for any final conclusion.

Of the several (five, according to Lysenko) phases in the development cycle, only three have so far been clearly identified. The first phase and second phase cover the period from germination to inflorescence, and the third phase, first noted by Kirichenko [1934], covers the period of the production of the gametes. The requirement of the first phase is specific temperature, of the second phase, temperature and photoperiod, and of the third phase, photoperiod. Thus, if the low temperature requirement of the first phase of winter wheat be lacking, the plant will not be able to advance to the inflorescence stage. But Purvis and Gregory [1937] have shown that preliminary short-day treatment, without low temperature, will enable winter

rye to advance to the reproductive stage, and that low temperature of germination cannot be regarded as the initiating factor in flower production but is rather an accelerator.

The vernalization experiments with mustard to be described in this paper corroborate the conclusion that germination at low temperature acts as an accelerator. Vernalization technique depends on the discovery of the principle that as soon as the growth of the dormant embryo starts, the seed can be treated, from a physiological point of view, as a growing plant. In other words, similar development processes will occur whether the environmental requirements of temperature and light are pre-supplied to the germinated seeds and seedlings before they are sown or transplanted, or whether the plants obtain these under natural conditions in an advanced stage of their vegetative growth. In other words, by using pre-treated seeds of certain crops it is possible to raise these crops in a region or during a given season in which the necessary environmental factors are normally lacking. In discussing the possibilities of vernalized seeds for Indian agriculture, however, it has been shown [Sen, 1939] that except for transplanted crops the many theoretical possibilities of pre-supply of environmental factors are limited practically to the supply of low temperature.

Lysenko maintains that 'the processes conditioning the sexual reproduction of cereals may occur not only in growing plants, but also in a seed with an embryo which has just commenced development but not broken the seed-coat' [Imp. Bur. of Pl. Genetics, 1935]. So far as we are aware, however, all vernalization experiments described in the literature have been carried out with chilled seedlings—for germinated seeds during the period of chilling normally develop into seedlings—and not chilled seeds with intact seed-coats. In the case of Gramineae (wheat, oat, barley, etc.) seeds with emerged coleoptile and several roots can be dried and successfully re-germinated, but drying is fatal in the case of mustard seeds with emerged radicles. In our preliminary report on vernalization of mustard [Sen and Chakravarti, 1938], experiments have been described which show that, (a) plants from chilled seeds—alike those which sprout during the period of chilling and those with intact seed-coat (unsplit seeds)—flower significantly earlier than plants from untreated control seeds, (b) for the same dose of chilling, the earliness in flowering is greater in plants from sprouted vernalized seeds, and (c) despite greater earliness that can be obtained from sprouted vernalized seeds, only unsplit vernalized seeds of mustard offer practical agricultural possibilities, since the sprouted chilled seeds have to be sown with great care for the reason that drying is fatal for them, while, on the other hand, unsplit seeds can be dried and stored without any loss of subsequent germinating capacity.

The results of the past four years' vernalization experiments with mustard at Almora (United Provinces) are described in the present paper. Most of the experiments were carried out with mustard Type 27 from New Delhi, but vernalization responses of mustard Types 9 and 11 from Cawnpore, and of yellow *sarson* and *raya* O.B/I from Lyallpur, have also been observed.

Working with winter wheat, Lojkin [1936] found that drying induced devernalization, and Gregory and Purvis [1938] observed similar reversal of vernalization induced by drying of vernalized grains of winter rye. Our

first problem, therefore, after our preliminary experiments [Sen and Chakravarti, 1938], was to find out whether vernalized unsplit seeds of mustard, when dried and stored for a minimum period necessary for the practical requirements of distribution and sowing, would retain unimpaired the effect of chilling. After the encouraging result of the first experiment of 1938, which showed that drying of unsplit chilled seeds up to 9 days—a likely minimum period required for distribution and sowing—did not impair the induced vernalization, experiments were undertaken to find out : (1) The optimum conditions and period of chilling necessary to induce maximum vernalization in unsplit chilled seeds of mustard. (2) Vernalization response of different strains of mustard. (3) Effect of vernalization on the progeny. (4) Period for which unsplit chilled seeds could be dried and stored without any loss of induced vernalization. (5) Effect of after-sowing temperature and day-length on the vegetative period of plants from control and vernalized seeds.

#### MATERIAL AND METHOD

The technique of vernalization previously described by Sen and Chakravarti [1938] has been found to be very satisfactory for small samples of seeds. For vernalizing larger samples necessary modifications were introduced, particularly in regard to the containers of seeds and provision for absorption of  $\text{CO}_2$  from the respiring seeds. The seeds to be chilled are first soaked under excess of water to make them absorb about 60 per cent of their weight of water, which generally takes six to eight hours, according to the room temperature. After removal of excess water by spreading the seeds over several layers of absorbent cloth, they are put in muslin bags or unglazed porcelain pots of suitable sizes and are then placed inside the moist-chamber of the chilling-cabinet.

Any watertight box of required dimensions with removable lid can be used for a moist-chamber. When boxes of thin wood are used, they should be thoroughly asphalted inside and out. The inside of the box is lined with blotting paper and sufficient water is placed at the bottom of the box to maintain the absorbent lining moist throughout the period of chilling. For absorption of  $\text{CO}_2$ , a concentrated solution of KOH is kept at the bottom of the box in a large, flat porcelain dish, the rim of which is previously paraffined to prevent creeping of KOH solution. A removable thick wire-net frame is placed over the KOH dish to protect the seeds against any accidental contact with the solution. Seeds in bags are suspended from hooks screwed on to the removable lid of the box, care being taken to see that the suspended bags do not touch the wire-net guard, or the moist blotting-paper lining of the box. When unglazed porcelain pots are used as seed containers, they are placed on the wire-net guard above the KOH solution. From daily readings of the maximum-minimum thermometer, the temperature range to which the seeds are subjected is recorded. Obviously, from these readings no definite idea is obtainable about the duration of the recorded temperatures each day.

#### *Chilling-cabinet*

An electrically operated cabinet of the Frigidaire type with an automatic device for maintaining a constant low temperature is undoubtedly the most

suitable appliance for chilling seeds. Since Almora has no electric supply, we used a kerosene-operated Electrolux for our experiments. An ordinary ice-box can, however, be used for chilling seeds, and when the low temperature required is not below 5°C. and only small samples of seeds are to be chilled, even a wide-mouthed thermos-flask can be used very successfully for chilling seeds in the following way. The thermos-flask is half-filled with freezing mixture and the soaked seeds are hung in a muslin bag from a hook screwed on the underside of the cork stopper of the flask. The process of daily renewal of the freezing mixture insures the necessary removal of CO<sub>2</sub> and a supply of fresh air. Additional moisture when required can be given to the seeds by dipping the bags in ice-cold water—the excess water automatically drips down into the flask. As will be seen later, mustard seeds thus chilled in a thermos-flask gave excellent results.

After the required periods of chilling, the sprouted mustard seeds are discarded and the unsplit seeds are washed and dried at room temperature till they attain a constant weight. The period varies from three to five days, according to the season. The seeds are then packed in a sealed container and stored inside the Electrolux.

Sowings were done both in pots filled with thoroughly sifted garden soil and in well-prepared field plots. When there were only two variables, i.e. one treatment each of vernalized and control seeds, they were sown in two halves of the same pot. Four plants (two of each) were grown in one pot (9 in. diameter). For preliminary trials two or three pots were used for each sowing. In final trials four pots for each variable were used. For field-plot trials four to six replications were used. To avoid errors of observation and possible injury to the growing point involved in determining the date of emergence of the first flower-buds, the date of the opening of the first flower—a strikingly visible phenomenon—was arbitrarily taken as the end of the vegetative period. The observed percentage of shortening of the vegetative periods (from sowing to opening of first flower) of plants from chilled seeds compared to those of control plants was taken as the measure of the vernalization response.

## EXPERIMENTS

### EXPERIMENT 1

As has already been stated, the first experiment carried out in 1938 was to determine whether chilled unsplit seeds of mustard when dried for a period of about a week would at all retain the induced vernalization. This experiment being of a preliminary nature, seeds from a batch chilled for 30 days were sown : (i) immediately after removal from chilling cabinet, on May 26, 1938 ; (ii) after drying for three days, on May 29 ; (iii) after drying for seven days, on June 2 ; and (iv) on June 4, after drying for nine days. The first three sowings were in pots and the fourth, replicated four times, was in well-prepared small garden plots. The control seeds used for the first sowing were previously soaked under water for six hours, and for the rest of the sowings, ordinary dried seeds were used. Control and vernalized seeds were sown in the two halves of each pot.

It will be seen from the data summarized in Table I that in all the four sowings, plants from unsplit chilled seeds flowered significantly earlier than

those from control seeds grown under similar after-sowing conditions. The vegetative periods of plants from V-seeds in all the three pot sowings were similar. The greater percentage of shortening observed in the first sowing (fresh chilled seeds) was due to the increased vegetative period of the plants from the C-seeds. From this experiment the conclusion is justified that unsplit chilled seeds of mustard when dried up to nine days—the likely minimum period required for distribution of seeds—will produce plants which will flower earlier than the untreated control.

TABLE I

*Vegetative period of mustard plants : C, from control seeds, and V, from vernalized unsplit seeds*

(Period of chilling, 30 days)

Sowing date (1938)	Average vegetative period (days)*	Period of drying of V-seeds (days)	Shortening of vegetative period of V-plants (per cent)
26/5 . . .	C—47.0 ± 2.05 (3) V—35.0 ± 0.75 (5)	0	25.5
29/5 . . .	C—41.8 ± 0.69 (10) V—36.0 ± 0.63 (16)	3	13.9
2/6 . . .	C—42.6 ± 1.06 (13) V—35.0 ± 1.2 (12)	7	17.8
4/6** . . .	C—53.6 ± 1.39 (37) V—38.7 ± 0.73 (40)	9	27.7

\* In this and in subsequent tables, figures within parantheses indicate the number of plants the mean of which is given.

\*\* Sown in small field plots.

## EXPERIMENT 2

To determine the period of chilling required for inducing maximum vernalization in unsplit chilled seeds, different batches of seeds were placed in the chilling-cabinet on appropriate dates to obtain, on July 1, 1938, batches of seeds chilled for eight, six, four and three weeks, respectively. These were all dried for 10 days and were sown, along with the controls, in four pots each on July 11, 1938. On this date batches of seeds chilled for four weeks but dried for 23 and 45 days were also available, and they were also sown in two pots each, to find out the effect of prolonged drying of unsplit chilled seeds.

The observed results are summarized in Table II. It will be seen that in all treatments plants from V-seeds flowered significantly earlier than those from C-seeds. The maximum earliness observed was from seeds chilled for six weeks (Plate I, fig. 2). The difference observed between treatments of eight, four and three weeks' chilling is not statistically significant, but the vegetative period of plants chilled for four weeks differs significantly only

from those of six weeks' chilling and not from eight and three weeks' chilling. Therefore, it was tentatively assumed that chilling for six weeks would induce maximum vernalization in unsplit seeds. It will also be seen that there is no significant difference in the vegetative periods of plants from seeds chilled for four weeks but dried for 10, 23 and 45 days. The fact that the seeds chilled for six weeks showed a higher degree of vernalization compared to those chilled for eight weeks clearly indicated that either, (i) the conditions of chilling were the optimum in the batch chilled for six weeks, or (ii) chilling beyond six weeks had induced devernalization. Therefore in 1939 investigations were undertaken to find out the optimum chilling conditions and also the effect of prolonged chilling.

TABLE II

*Effects of different periods of chilling and drying*

(Sowing date, July 11, 1938)

Period of chilling (weeks)	Period of drying (days)	Vegetative period (days)	Shortening of vegetative period in V-plants (per cent)
Nil (control) .	..	44.9 ± 1.17 (15)	..
3 . . .	10	37.0 ± 0.73 (15)	17.6
4 . . .	0	41.3 ± 1.5 (7)	8.0
4 . . .	10	37.8 ± 0.85 (16)	15.8
4 . . .	23	38.0 ± 1.17 (8)	15.3
4 . . .	45	39.5 ± 0.81 (8)	12.0
6 . . .	10	34.7 ± 0.96 (16)	22.7
8 . . .	10	37.0 ± 0.97 (15)	17.6

*Analysis of variance*

Due to	D. F.	Sum of sq.	Mean sq.	Ratio observed
Between treatment .	7	984.57	140.65	10.05 **
Within treatment . .	92	1286.00	13.98	
Total .	99	2270.57	..	..

S. E. per plant 3.74

\*\*Significant at 1 per cent level

*Optimum chilling conditions*

In the case of winter wheat, it has been shown by Lojkin [1936] that the degree of vernalization induced increases with a greater rate of life activity within the seeds during the process of chilling. Sen and Chakravarti [1938] have also found this to be equally true in the case of mustard. Working with excised embryo of winter rye, Gregory and Purvis [1938] have clearly demonstrated that the reactions involved in the process of vernalization are localized in the embryo of the seeds. Obviously, the rate of life activity of the embryos of seeds which sprout during the process of chilling must be greater than that of seeds which remain unsplit. The fact that when a batch of soaked seeds of mustard is chilled varying proportions of both sprouted and unsplit seeds are obtained, indicates that, (i) all the seeds of a given sample are not similar in regard to their speed of germination, or (ii) that during the process of chilling all the seeds are not subjected to identical environmental factors of temperature, moisture supply and oxygen tension, or (iii) a combination of both (i) and (ii). That the speed of germination of individual seeds of a given sample of mustard varies, is seen from the fact that even at room temperature (20°-25°C.) when soaked seeds are spread over moist blotting paper in covered petri dishes in a single layer in batches of 50, it generally takes 14-15 hours from the sprouting of the first seeds in each batch until all the seeds in the batch have sprouted. At lower temperature (5°-10°C.) this period is increased on an average to 15 days. Since we have been able to recover unsplit mustard seeds from samples chilled for 365 days, it is evident that germination speed of individual seeds is not similar, and seeds densely piled in bags for chilling are obviously not subjected to uniform environmental factors. No attempt has been made to overcome this problem, since it is due to this very lack of uniformity, alike of the speed of germination and of the environmental conditions, that we have been able to recover unsplit chilled seeds. Furthermore, the seeds which sprout during the process of chilling offer the only visible indication that life activity is being maintained in the samples as a whole. Experiments have been undertaken, however, to find out the optimum temperature range and moisture supply for obtaining maximally vernalized unsplit seeds.

**EXPERIMENT 3**

For lack of an automatic device for maintaining different low temperature ranges, advantage was taken of the definite temperature gradient which exists inside a kerosene-operated Electrolux. Three different batches of mustard seeds (2.5 gm. each), after being soaked under water for six hours, were kept on the three shelves of the Electrolux, in three moist-chambers within which high humidity was maintained. From the daily record of the three maximum-minimum thermometers kept on the three shelves, the average temperature for the period of chilling (31 days) was obtained. The chilled unsplit seeds were dried at room temperature till they attained a constant weight, and each sample was weighed to determine the percentage of recovery of unsplit seeds. The results of sowings of these seeds on September 11, 1939, are given in Table III. The statistical analysis of the data shows that compared to the plants from the three samples of unsplit vernalized seeds,

chilled at different temperature ranges, the plants from untreated control seeds flowered significantly later, and that the differences observed in the vegetative periods of plants from different batches of vernalized unsplit seeds were not significant. Thus it would appear that a temperature range between 2° and 12°C. can be successfully used for chilling mustard seeds, but the higher the temperature the smaller will be the recovery of unsplit chilled seeds.

TABLE III

*Effect of chilling at different temperature ranges with similar moisture supply, and percentage of recovery of unsplit chilled seeds*

(Sowing date, September 11, 1939)

Mean temperature (°C.)	Vegetative period (days)	Recovery of un- split seeds (per cent)	Shortening of vegetative period of V-plants
Control . . . . .	64.4 ± 2.39 (10)	..	....
Top shelf 3.5—12 .	45.6 ± 3.10 (10)	7.2	18.8 days, or 29.2 per cent
Middle shelf 2—10 .	45.4 ± 2.42 (9)	14.4	19.0 days, or 29.5 per cent
Bottom shelf 0—6 .	47.3 ± 2.73 (8)	32.0	17.1 days, or 26.5 per cent

*Analysis of variance*

due to	D. F.	Sum of sq.	Mean sq.	Ratio observed
treatment .	3	2576.47	858.82	11.49 **
block .	33	2464.80	74.69	
Total .	36	5041.27	..	..

S. E. per plant 8.64

\*\* Significant at 1 per cent level

#### EXPERIMENT 4

Under otherwise similar conditions, the rate of life activity of the embryo of mustard seeds increases, within limits, with increased supply of moisture, and therefore for the same dose of chilling the vernalization induced will vary according to the moisture supply. But to obtain vernalized unsplit seeds the embryo must not be allowed to grow beyond the elastic limit of the seed-coat. Though it has not yet been possible for us to determine the critical stage of

growth of the embryo at which the seed-coat will burst, some rough estimate has been obtained about the effect of high and low humidity supply on induced vernalization under similar low temperature range. The variation of moisture supply was obtained by using different seed containers. For this experiment batches of 250 gm. of seeds were used for each treatment. For low humidity, two batches of previously soaked seeds were placed in unglazed porcelain pots and no additional water was given during the period of chilling; for high humidity, five batches of seeds were put in muslin bags and every week the bags were dipped in ice-cold water. Both sets of seeds were kept in the same moist-chamber. The seeds in unglazed porcelain pots were placed on top of the wire-net guard of the moist-chamber, and the muslin bags were hung from the hooks attached to the removable lid of the moist-chamber. The two batches of seeds in unglazed porcelain pots were chilled for longer periods than any used for batches in muslin bags. The various batches were placed in the chilling-cabinet at appropriate dates so that by September 22, 1939, the samples subjected to low humidity had been chilled for 14 and 12 weeks, and those subjected to high humidity for ten, eight, six, four and two weeks. After removing the sprouted seeds, the unsplit chilled seeds of all the batches were dried at room temperature for 10 days and weighed. These seven samples of chilled unsplit seeds were sown along with the untreated control seeds in well-prepared field plots. Four replications were used for each of the variables. The vernalization response of the different samples of chilled seeds and the percentage of recovery of the unsplit seeds from each sample are given in Table IV, and further details of this sowing are given later (Table IX) along with the statistical analysis of the data.

TABLE IV

*Vernalization response of unsplit seeds chilled at low and high humidity and the recovery percentage of unsplit seeds*  
(Sowing date, October 2, 1939)

Period of chilling  (weeks)	Humidity	Vernalization response  (per cent)	Recovery of unsplit seeds  (per cent)
2 . . . .	High	10.05	78.0
4 . . . .	„	14.24	58.2
6 . . . .	„	30.26	33.5
8 . . . .	„	28.46	40.0
10 . . . .	„	31.37	34.0
12 . . . .	Low	13.86	60.0
14 . . . .	„	25.16	67.0

The observed differences in vernalization response of samples chilled for six, eight and ten weeks with high humidity and 14 weeks with low humidity are not statistically significant. This shows that chilling for six weeks under optimum conditions induces maximum vernalization in unsplit seeds and that chilling under similar conditions for 10 weeks does not induce any devernization. Though chilling with high moisture supply for only six weeks induces maximum vernalization comparable to that induced by chilling for 14 weeks with low moisture supply, the percentage of recovery of unsplit seeds in the case of low humidity treatment is nearly double the figure for high humidity treatment.

#### EXPERIMENT 5

The conclusions of experiments 3 and 4 explain why the unsplit seeds chilled in a thermos-flask were maximally vernalized. An experiment was undertaken earlier to explore the possibility of utilizing simple devices for vernalizing mustard seeds. A temperature range of  $4^{\circ}$ — $8^{\circ}$ C. and maximum humidity can be obtained for chilling seeds inside a wide-mouthed thermos-flask (such as is used commonly as a food-jar) half filled with freezing mixture. A batch of mustard seeds previously soaked under water for six hours was chilled in a thermos-flask for 52 days. The freezing mixture was renewed daily, thus assuring an adequate supply of oxygen for the seeds. The unsplit seeds chilled in a thermos-flask, after being dried for three days, were sown in pots on April 24, 1939, along with maximally vernalized unsplit seeds (chilled for 167 days) and the untreated control. The vegetative period of plants from control seeds was  $41.5 \pm 1.95$  days (mean of four plants), while the periods of plants from seeds chilled in the thermos-flask for 52 days and chilled in the Electrolux for 167 days were found to be similar, being  $31.0 \pm 0.79$  days (mean of five plants) and  $31.9 \pm 0.54$  (mean of eight plants), respectively.

Another preliminary experiment was undertaken to find out whether the ground temperature of Almora ( $2^{\circ}$ — $8^{\circ}$ C.) could be used during winter for chilling seeds as a cheap method of vernalization. Chilling for 35 days under frost-covered ground produced a significant vernalization response, but the results showed that the seeds were not completely vernalized, as the shortening of the vegetative period from maximally vernalized seeds, sown on the same date, was 25.6 per cent, while those of plants from seeds chilled by the ground temperature for 35 days was only 12.7 per cent. From this experiment it is evident that at least partial vernalization can be obtained without the cost of operating a chilling-cabinet. The possibility of obtaining maximally vernalized unsplit mustard seeds is being explored.

#### EXPERIMENT 6

In the case of winter wheat it is reported [Imp. Bur. of Pl. Genetics, 1935] that seeds can be vernalized by instalment. For instance, instead of chilling continuously for 50 days, seeds may be chilled for 40 days, kept in a dry state until required, and then given a further period of chilling for 10 days before sowing. On the other hand, Gregory and Purvis [1938] have shown that 'as far as tendency to flower is concerned, the vernalized seeds of rye dried for 20 weeks are identical with unvernized control.' In

the case of mustard, it has already been shown (experiment 2) that drying of unsplit chilled seeds up to 45 days does not affect the induced vernalization. The following experiment was undertaken to find out : (i) whether drying of partially vernalized unsplit mustard seeds for a period of more than 20 weeks would induce complete devernalization ; (ii) whether partially vernalized unsplit mustard seeds could be re-chilled to induce maximum vernalization ; and (iii) whether prolonged chilling would induce devernalization.

Plants from a batch of seeds chilled for six weeks from August 23 to October 4, 1939, dried for 10 days and sown in a small field plot showed a significant shortening of the vegetative period of only 7.2 per cent, a percentage considerably lower than could be expected from maximally vernalized unsplit seeds. This batch of seeds was stored in the Electrolux in a sealed bottle. On December 18, 1938, a sample from this batch of stored chilled seeds was soaked under water for six hours and re-chilled in the usual way till March 4, 1939. Still another batch of seeds was chilled uninterruptedly from October 25, 1938 to March 4, 1939. Both the re-chilled and continuously chilled unsplit seeds were dried for seven days. Thus, on March 11, 1939, the four batches of seeds sown were : (1) chilled for six weeks, dried for 158 days ; (2) chilled first for six weeks and then, after drying for 74 days, re-chilled for a further period of 77 days (the combined period of chilling being 119 days) and re-dried for seven days ; (3) chilled continuously for 129 days and dried for seven days ; and (4) control. From the observed data given in Table V it will be seen that : (i) incompletely vernalized unsplit seeds of mustard when dried and stored for 158 days can retain the effect of chilling, for they produced plants which flowered significantly earlier than the control plants ; (ii) incompletely vernalized unsplit seeds can be re-chilled after a period of drying for 74 days, to induce maximum vernalization, for the difference observed between Nos. 1 and 2 is statistically significant ; (iii) continuous chilling for a period of 129 days did not produce any injurious effect on the unsplit seeds, at least as far as the vernalizing reactions were concerned.

TABLE V

*Effects of re-chilling incompletely vernalized unsplit seeds and of continuous prolonged chilling on induced vernalization*

(Sowing date, March 11, 1939)

Nos.	Treatment of seeds	Vegetative period	Shortening of vegetative period in V-plants (per cent)
1	Chilled 6 weeks, dried 158 days .	41.77 ± 0.46 (13)	4.5
2	Chilled 6 weeks, after drying 74 days re-chilled 77 days and then dried 7 days	38.14 ± 0.25 (14)	12.8
3	Continuously chilled 129 days .	38.17 ± 0.56 (12)	12.7
4	Control . . . . .	43.75 ± 0.56 (12)	..

These results indicate that it is possible to utilize any natural winter ground temperature ranging from 1° to 12°C. for vernalization of mustard seeds, since even if the cold temperature available in any given region be for a period not long enough to induce maximum vernalization, the partially vernalized unsplit seeds can be dried and stored for subsequent re-chilling by artificial low temperature at a convenient date.

#### *Vernalization response of different strains of mustard*

Preliminary experiments were undertaken to find out whether strains of mustard other than Type 27 would also respond to vernalization. Two strains of mustard from Cawnpore, Types 9 and 11, two strains from Lyallpur, *raya* O.B/I, and yellow *sarson*, were tried. It was found that unsplit chilled seeds of all these strains of mustard produced plants which flowered earlier than the plants from untreated control seeds. Compared to C 11, the vernalization response of C 9 was considerably greater. In the Lyallpur strains, in a sowing of September 6, 1938, the percentage of earliness observed in the opening of the first flower of plants from unsplit seeds of *raya* O.B/I and of yellow *sarson*, both chilled for 30 days and dried for 10 days, was 19·8 and 39·7, respectively.

#### EXPERIMENT 7

A sowing was undertaken to find out the comparative vernalization responses of mustard Type 27, C 11 and C 9. Samples of these strains were chilled for 52 days, and after drying at room temperature for four days the unsplit chilled seeds were sown, along with their respective controls, on August 11, 1939. From the results summarized in Table VI, it will be seen that with similar pre-chilling treatment of seeds and under similar after-sowing environmental conditions the percentage of shortening was the greatest in plants from chilled unsplit seeds of Type C 9 and lowest in Type C 11.

TABLE VI

#### *Comparative vernalization responses of mustard Type 27, C 9 and C 11*

(All chilled for 52 days, dried for 4 days and sown on August 11, 1939)

Strain	Vegetative period (days)	Shortening of vegetative period in V-plants
C 11	C—40·16±0·64 (12) V—31·82±1·3 (11)	8·34 days or 20·76 per cent
Type 27 . . . . .	C—59·9±2·06 (10) V—37·55±0·92 (9)	22·35 days or 37·31 per cent
C 9 . . . . .	C—61·44±2·93 (9) V—30·18±1·53 (11)	31·26 days or 50·88 per cent

*Effect of vernalization on the progeny*

## EXPERIMENT 8

To find out whether the effect of vernalization is transmitted to the progeny, four different sowings were undertaken, and the vegetative periods of plants from seeds collected for three successive generations of control and vernalized seeds were observed. In these observations it was assumed that if the effect induced by chilling of seeds is transmitted to the progeny, then the plants from seeds collected from the very first generation of vernalized seeds would produce at least partially vernalized seeds. If, however, the effect transmitted to the progeny be of an undetectable intensity in the first generation, then vernalization of the progeny of vernalized seeds for three successive generations might be expected to give some visible indications. The first sowing was undertaken to collect the progenies of control and vernalized seeds. In the second sowing, the progenies of the control and vernalized seeds were vernalized and sown along with their respective untreated controls. This process was continued for the third and fourth sowings. The observed vegetative periods in all these sowings are given in Table VII.

TABLE VII

*Vegetative periods of plants from three successive generations of vernalized and control seeds*

No.	Seed stock	Sowing date	Period of chilling (days)	Mean vegetative period (days)	Significance
1	Original stock	June 4, 1938	..	C—53.6 ± 1.39 (37)	
			30	V—38.7 ± 0.75 (40)	**
2	Progeny of 1	May 15, 1939	..	cC—41.7 ± 1.50 (12)	
			..	vC—41.0 ± 0.52 (12)	Not sig.
			50	cV—28.4 ± 0.64 (11)	
			50	vV—30.3 ± 0.51 (12)	Do.
3	Progeny of 2	Sept. 2, 1939	..	ccC—71.3 ± 2.53 (7)	
			..	vvC—74.7 ± 3.45 (4)	Do.
			30	ccV—34.8 ± 2.21 (5)	
			30	vvV—35.2 ± 1.72 (5)	Do.
4	Progeny of 3	June 5, 1940	..	cccC—40.4 ± 0.9 (8)	
			..	vvvC—47.1 ± 0.7 (8)	**
			51	cccV—33.8 ± 1.39 (7)	
			51	vvvV—39.6 ± 1.62 (8)	**

\*\* Significant at 1 per cent level

For abbreviation, the generations of the seeds used are indicated by small letters (c, control, and v, vernalized). The particular treatment given in each sowing, i.e. control or vernalized, is indicated by capital letters (C, untreated control, and V, vernalized). Thus, for example, control seeds used

for the third sowing are indicated as ccC and vvC. They are untreated progenies of the second generation of control and vernalized seeds. Similarly in the fourth sowing, vernalized seeds of the third generations are indicated as cccV and vvV.

Except in the third sowing only, unsplit vernalized seeds were used for the vernalization test. Sprouted vernalized seeds had to be used for the third sowing because not only was the quantity of seeds we could collect from the second sowing very small, but the quality of the seeds appeared to be poor as well, and we could not be sure that unsplit chilled seeds of this sample would be viable. Both the sprouted control and vernalized seeds of the third sowing produced normal seeds, however, which were used for the fourth sowing, when again unsplit chilled seeds were used. From Table VII it will be seen that in sowings 2 and 3 progenies of control and vernalized seeds, alike untreated and vernalized, produced plants with similar vegetative periods, since the observed differences are not statistically significant. In sowing 4, however, the differences observed in the vegetative periods of cccC- and vvC-plants and cccV- and vvV-plants are statistically significant, but it was the ccc-seeds which produced plants which flowered earlier than those from vvV-seeds. Thus, it can be concluded that the effect of vernalization, as far as earliness in flowering is concerned, is not transmitted in the case of mustard Type 27 up to the third generation. The significant earliness observed in flowering of plants from ccc-seeds was due not to any cumulative inheritance nor to deterioration of vvV-seeds but, as will be seen from the following preliminary experiments, to the lower temperature range during the period of development and maturity of the seeds of ccC-plants, the first flowers of which opened from November 2 to November 24, while the corresponding period of the vvV-plants was from October 2 to October 14.

#### EXPERIMENT 9

It has been observed by Kostjucenko and Zarubalio [1937] that wheat seeds which develop and mature under low temperature become naturally vernalized. Gregory and Purvis [1938] actually produced vernalized winter rye seeds by chilling the ear. Mustard is a winter crop. In comparison with the Delhi region, Almora has a much colder winter, and as has already been shown the winter ground temperature of Almora can in fact be utilized for vernalization of mustard (Experiment 5). If low temperature during seed reproduction can induce at least partial vernalization, then, (i) untreated mustard seeds of the normal Almora harvest would be expected to produce plants which would flower earlier than those from the normal Delhi harvest, and (ii) Almora summer temperature being considerably higher than that of the Delhi winter, seeds reproduced in Almora from off-season sowings would be expected to produce plants which would flower later than plants from the normal Delhi harvest. The results of different sowings given in Table VIII indicate that low temperature during seed ripening will produce partially vernalized seeds; for in sowings Nos. 1, 2 and 3 the seeds from normal Almora harvest produced plants which flowered significantly earlier than those from the Delhi normal harvest, but in sowing 4, Almora summer seeds produced plants which flowered later than those from the normal Delhi harvest.

TABLE VIII

*Vegetative periods of plants from seeds reproduced under different temperature ranges in Delhi and Almora*

Nos.	Date of sowing	Seeds	Vegetative period (days)	Earliness (days)	Significance
1	Sept. 26, 1940	Normal harvest	Delhi $-60.77 \pm 1.4$ (13) Almora $-55.1 \pm 1.46$ (11)	5.67	**
2	Feb. 25, 1941	„	Delhi $-46.58 \pm 0.36$ (12) Almora $-44.72 \pm 0.61$ (11)	1.86	*
3	June 14, 1941	„	Delhi $-53.71 \pm 1.67$ (14) Almora $-48.75 \pm 1.41$ (12)	4.96	*
4	Sept. 11, 1940	Summer harvest Normal harvest	Almora $-62.7 \pm 1.4$ (12) Delhi $-53.2 \pm 0.58$ (9)	9.5	**

\* Significant at 5 per cent level ; \*\* Significant at 1 per cent level

*Vegetative period of plants from vernalized seeds under field conditions*

The observations so far described were carried out mostly in pot cultures. Author series of experiments was undertaken to find out the shortening of the vegetative period that can be obtained by the use of vernalized mustard seeds grown under field conditions. Three strains of mustard—Type 27, C 11 and C 9—were used. The effect of different periods of chilling and also the effect of different periods of drying of unsplit vernalized mustard seeds were observed. With the co-operation of Dr T. S. Sabnis and of Dr B. P. Pal vernalized unsplit seeds sent from Almora by post were given field-plot trials in Cawnpore and New Delhi respectively. In Cawnpore all the three strains were tried and in New Delhi only Type 27 was used.

#### EXPERIMENT 10

The effect of different periods of chilling on the degree of vernalization induced was observed by simultaneous sowings of different batches of seeds chilled for different periods. Seeds of mustard Type 27, C 11 and C 9 were placed in the chilling-cabinet on appropriate dates so that on September 23, 1939, various batches of Type 27 seeds were obtained which had been chilled for 14, 12, 10, 8, 6, 4 and 2 weeks, respectively, and batches of C 11 and C 9 seeds, which had been chilled for 14, 10, 6 and 2 weeks. All unsplit chilled seeds of all batches of each strain were dried for the same period before they were sown along with their untreated controls in well-prepared field plots. Four replications were used for each treatment. The results obtained are given in Tables IX, X, and XI. It will be seen from the statistical analysis of the data that in Type 27 and in C 11 chilling for six weeks induces maximum vernalization in unsplit seeds, and further increase in the period of

chilling does not induce any higher degree of vernalization, neither any devernialization (Tables IX and X). In the case of C 9, chilling for a period longer than six weeks is necessary to induce maximum vernalization in unsplit seeds, since the vegetative period observed in plants chilled for 10 weeks is significantly shorter than in those from seeds chilled for six weeks (Table XI). The increased vegetative period observed (Table IX) in plants from seeds chilled for 12 weeks compared to those from seeds chilled for 6, 8, 10 and 14 weeks is due, as has already been explained (Experiment 4), to the reduced moisture supply.

TABLE IX

*Effect of different periods of chilling (mustard Type 27)*

(Unsplit chilled seeds dried for 9 days, sown October 2, 1939)

Period of chilling (weeks)	Vegetative period (days)	Earliness.	
		Days	Percentage
Control . . . . .	80.96 $\pm$ 1.27 (47)	..	..
2 . . . . .	72.82 $\pm$ 1.38 (50)	8.14	10.1
4 . . . . .	69.43 $\pm$ 1.73 (46)	11.53	14.2
6 . . . . .	56.46 $\pm$ 1.06 (45)	24.5	30.3
8 . . . . .	57.92 $\pm$ 0.75 (48)	23.04	28.5
10 . . . . .	55.56 $\pm$ 0.88 (46)	25.4	31.4
12 . . . . .	69.74 $\pm$ 1.0 (47)	11.22	13.9
14 . . . . .	60.59 $\pm$ 1.37 (47)	20.37	25.2

*Analysis of variance*

Due to	D. F.	Sum of sq.	Mean sq.	Ratio observed
Treatment . . . . .	7	27565.93	3937.99	23.85 **
Block . . . . .	3	1846.16		
Error . . . . .	21	3466.44	165.07	
Total . . . . .	31	32878.53		

S. E. per plant 12.85

\*\* Significant at 1 per cent level

TABLE X

*Effect of different periods of chilling (mustard C 11)*  
(Unsplit chilled seeds dried for 16 days, sown October 9, 1939)

Period of chilling (weeks)	Vegetative period (days)	Earliness	
		Days	Percentage
Control . . . . .	$85.43 \pm 3.16$ (23)	..	..
2 . . . . .	$77.59 \pm 3.17$ (22)	7.84	9.17
6 . . . . .	$65.09 \pm 5.19$ (22)	20.34	23.81
10 . . . . .	$68.25 \pm 4.46$ (24)	17.18	20.11
14 . . . . .	$67.67 \pm 3.82$ (22)	17.76	20.79

*Analysis of variance*

Due to	D. F.	Sum of sq.	Mean sq.	Ratio observed
Treatment . . . . .	4	6557.11	1639.27	7.46 **
Block . . . . .	3	1139.73		
Error . . . . .	12	2634.08	219.5	
Total . . . . .	19	10330.92	..	..

S. E. per plant 14.8

\*\* Significant at 1 per cent level

TABLE XI

*Effect of different periods of chilling (mustard C 9)*  
(Unsplit chilled seeds dried for 16 days, sown October 9, 1939)

Period of chilling (weeks)	Vegetative period (days)	Earliness	
		Days	Percentage
Control . . . . .	$94.44 \pm 4.8$ (25)	..	..
2 . . . . .	$80.35 \pm 2.71$ (30)	14.09	14.9
6 . . . . .	$73.19 \pm 4.29$ (26)	21.25	22.5
10 . . . . .	$65.32 \pm 3.33$ (28)	29.12	30.8
14 . . . . .	$64.87 \pm 3.37$ (30)	29.57	31.3

*Analysis of variance*

Due to	D. F.	Sum of sq.	Mean sq.	Ratios observed
Treatment . . .	4	16152.65	4038.16	19.02 **
Block . . .	3	11583.78		
Error . . .	12	2547.75	212.31	
Total . . .	19	30284.18	..	..

S. E. per plant 14.56

\*\* Significant at 1 per cent level

For trials in Cawnpore (Plate I, fig. 3) and in New Delhi, unsplit vernalized seeds chilled for 14 weeks were sent. The shortening of the vegetative period of plants from vernalized seeds of different strains of mustard observed in different stations is given in Table XII. It will be seen that irrespective of the region, vernalized unsplit seeds produced plants with shorter vegetative period, but that the earliness observed varied according to the strain of mustard. The differences observed in the percentage of shortening of the vegetative periods of the plants from the same batches of vernalized and untreated seeds but grown in different regions must obviously be due to the after-sowing environmental factors of the regions concerned.

TABLE XII

*Vegetative periods of plants from control and vernalized seeds of different strains of mustard grown in different regions*

Strain	Sowing date	Station	Vegetative period (days)	Earliness	
				Days	Percentage
Type 27 . . .	2-10-39	Almora . . .	C—80.96 V—60.59	20.37	25.2
	17-10-39	Cawnpore . . .	C—65.0 V—52.0	13.0	20.0
	21-10-39	New Delhi . . .	C—88.03 V—78.38	9.65	10.9
C 11 . . .	9-10-39	Almora . . .	C—85.43 V—67.67	17.76	20.8
	17-10-39	Cawnpore . . .	C—48.0 V—41.0	7.0	14.6
C 9 . . .	9-10-39	Almora . . .	C—94.44 V—64.87	29.57	31.3
	17-10-39	Cawnpore . . .	C—63.0 V—46.0	17.0	27.0

# MUSTARD TYPE 27 FROM UNTREATED CONTROL AND VERNALIZED SEEDS

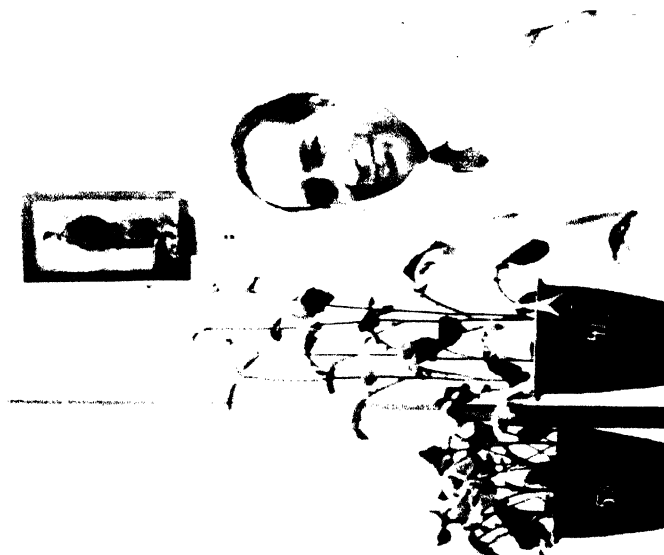


FIG. 1. Plants in pot 53 are from untreated control seeds and those in pot 41 are from unsplit seeds chilled for 50 days [ Seeds sown May 15, 1939, photographed June 14, 1939, Almora ]



FIG. 2. Plants in two pots in the middle are from control seeds ; those in two pots on the left, from seeds chilled for 8 weeks ; those in two pots on the right from seeds chilled for 6 weeks [ Seeds sown July 11, 1938, Almora ]



FIG. 3. Seeds sown August 11, 1939, photographed October 23, 1939, Cawnpore Agricultural Farm [ Vernalized unsplit seeds used were chilled for 77 days and dried for 8 days (T. S. Sabnis) ]

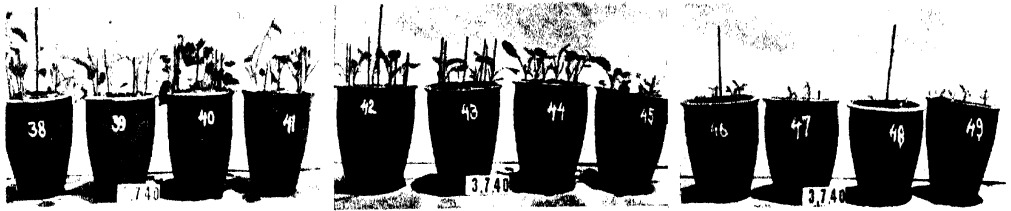


FIG. 1. Three sets of pots of mustard type 27 grown under different photoperiods.  
Photographed on July 3, 1940



FIG. 2. The same pots on July 17, 1940



FIG. 3. One pot each from the three different sets showing similar vegetative periods of C-plants under 14 hrs photoperiods and V-plants under 10 hrs photoperiod. Photographed July 27, 1940

[ Plants in left half of each pot are from maximally vernalized unsplit seeds, those on the right half, from untreated control seeds. Sown on June 6, 1940. Light treatment from June 11, 1940. Pots 38-41 had full day-length of 14 hrs, pots 42-45 had 11 hrs daylight for 3 weeks and full day-length afterwards, pots 46-49 had 8 hrs daylight for 3 weeks and full day-length afterwards. ]

*The effect of drying and storage on vernalized unsplit seeds*

For environmental studies, the complications due to low-temperature requirements of the first phase of development can be eliminated by the use of vernalized seeds. With the limited facilities at our disposal, the problem of supply of strictly comparable vernalized seeds for different seasonal sowings at first appeared formidable, since it was not possible to arrange throughout the year strictly controlled similar low-temperature range, moisture supply and oxygen tension for chilling. But the fact that even incompletely vernalized unsplit seeds when dried and stored for 158 days were found to retain the induced vernalization (Experiment 6) suggested the possibility of using the same batch of vernalized unsplit seeds for different seasonal sowings. If the plants from the same batch of vernalized unsplit seeds would show similar vegetative periods in similar sowing dates of two successive years, it could be assumed that, (a) the induced vernalization had remained unimpaired for the period, and (b) any observed variations in the vegetative periods in the intermediate sowings were due to changes in after-sowing temperature and day-length of the season. Therefore a long series of sowings from the same batch of vernalized seeds was undertaken, to find out the period for which vernalized unsplit seeds when dried and stored would retain the induced vernalization unimpaired.

**EXPERIMENT 11**

A batch of mustard seeds (Type 27) was chilled for 167 days, much longer than was necessary to induce maximum vernalization. The period was purposely prolonged to repeat the observation of Experiment 6, to find out whether prolonged chilling would be injurious to unsplit seeds. The seeds were placed in the moist-chamber of the Electrolux on October 18, 1938 and were removed on April 3, 1939. After being dried for three days at room temperature, the unsplit chilled seeds were put in cold storage in a sealed bottle. All sowings from this batch of vernalized seeds, along with the controls, were made in pots kept in the glass-house. A daily record of the maximum and minimum temperature of the glass-house was kept. The curve of the seasonal day-length of Almora was obtained from Dr L. A. Ramdas, of the India Meteorological Department, Poona. Readings of the dry-and-wet-bulb thermometer kept in the glass-house were taken every afternoon. The results of the 20 sowings spread over the period from April 24, 1939, to March 28, 1941, are summarized in Table XIII. For convenience the different sowings on similar dates of two successive years are grouped together. It will be seen that the total period of drying of vernalized unsplit seeds in sowings Nos. 1 and 9 were 21 and 387 days, respectively, and yet the observed vegetative periods of the plants were remarkably similar. The vegetative periods of plants from control seeds in these two sowings were also very similar. Therefore, it may be concluded that drying of vernalized unsplit seeds for a period of 387 days did not induce any devernalization.

But from the comparison of the data from sowings Nos. 2 and 11 of June 26, 1939, and of June 27, 1940, no definite conclusion about the condition of the V-seeds seems to be justified, in spite of the fact that the plants from these seeds had very similar vegetative periods. For a marked shortening of the

vegetative period of plants from C-seeds was observed in sowings of 1940, which obviously must have been due to more favourable after-sowing environmental conditions of the year. The lack of any observable effect of favourable after-sowing environmental factors upon the vegetative periods of plants from V-seeds might very possibly have been due to slight devernalization resulting from prolonged drying, or it might have been due to the fact that the vegetative period of 29.9 days observed in 1939 was already the minimum for plants from these seeds, and therefore no further diminution could be expected. Therefore, in the next sowing of this series an independent estimate was sought about the condition of this particular batch of V-seeds by sowing on the same date with them seeds from another batch of vernalized unsplit seeds chilled for 365 days but dried for seven days only. The results of sowing No. 12 indicate that the condition of the V-seeds chilled for 167 days and dried for 462 days was similar to that of seeds chilled for 365 days and dried only for seven days. In other words, the vernalization induced in seeds remained unimpaired even when the seeds were dried for 462 days. A similar test with seeds chilled for 365 days was undertaken in sowing No. 18, with similar verification. In all sowings under similar conditions, we have not found any batch of chilled unsplit seeds, including those chilled for 365 days, which produced plants with shorter vegetative period than that of plants from seeds chilled for 167 days.

To find out whether the capacity of unsplit vernalized seeds to withstand drying was equally true of other batches of maximally vernalized seeds chilled for a period shorter than 167 days, unsplit seeds from batch (a) chilled for 65 days was sown on June 14, 1941, together with batch (b) chilled for 365 days for comparison. On that date the period of drying and storage of a-seeds was 765 days and of b-seeds 348 days. The observed vegetative periods were  $31.75 \pm 0.82$  days (mean of 16 a-plants) and  $31.14 \pm 0.71$  days (mean of 7 b-plants). Thus, it is evident that maximally vernalized unsplit seeds of mustard Type 27 irrespective of period of chilling can withstand drying and storage and still retain the effect of vernalization for at least 765 days.

From the data given in Table XIII it can be concluded that : (1) in all the different sowings plants from the same batch of vernalized seeds flowered significantly earlier than those from untreated control seeds ; (2) an annual cyclic variation of the vegetative periods of plants alike from vernalized and control seeds, due to differing after-sowing environmental conditions of the seasons, can normally be expected ; (3) similar after-sowing environmental conditions affect the vegetative periods of C- and V-plants differently, since the shortest vegetative period of approximately 41 days observed in C-plants was in sowings of April and May of 1939 and 1940, while in the case of V-plants the shortest period of about 31 days was observed in sowings of April to August of both 1939 and 1940. The longest vegetative periods of C- and V-plants, however, were observed in sowings of October—December ; (4) maximally vernalized unsplit seeds of mustard when dried and stored for a period of 725 days retain the induced vernalization unimpaired. This last conclusion offered the most convenient solution of the problem of supply of strictly comparable vernalized seeds for environmental studies, for seeds from a batch of maximally vernalized seeds can be used for sowings spread over a period

TABLE XIII

*Vegetative period of plants from seeds chilled for 167 days and sown at different seasons of two successive years*

(Mustard Type 27)

No.	Sowing date	Vegetative periods (days)		Drying (days)	Earliness in V-plants	
		Control	Vernalized		Days	Per cent
	<i>April</i>					
1	24, 1939 . . . .	41.5 ± 1.95 (4)	31.9 ± 0.54 (8)	21	9.6	23.1
9	24, 1940 . . . .	41.03 ± 0.65 (15)	30.53 ± 0.41 (15)	387	10.5	25.6
	<i>May</i>					
10	22, 1940 . . . .	41.3 ± 0.73 (12)	30.0 ± 0.72 (10)	415	11.3	27.3
	<i>June</i>					
2	27, 1939 . . . .	54.3 ± 0.58 (3)	29.9 ± 1.48 (5)	85	24.4	44.9
11	29, 1940 . . . .	46.75 ± 0.92 (8)	29.5 ± 0.49 (11)	453	17.25	36.9
	<i>July</i>					
12	8, 1940 . . . .	47.18 ± 0.85 (11)	31.75 ± 0.64 (12)	462	15.43	32.7
	8, 1940 . . . .	(Chilled 365 days)	31.00 ± 0.92 (7)	7	16.18	34.3†
	<i>August</i>					
3	11, 1939 . . . .	59.9 ± 2.06 (10)	28.9 ± 0.79 (8)	130	31.0	51.7
13	12, 1940 . . . .	52.6 ± 1.28 (8)	32.1 ± 0.76 (6)	497	20.5	38.0
	<i>September</i>					
14	26, 1940 . . . .	60.77 ± 1.11 (13)	37.6 ± 0.58 (11)	542	23.17	38.1
	<i>October</i>					
4	11, 1939 . . . .	82.0 ± 1.6 (8)	50.0 ± 2.42 (8)	191	32.0	39.0
15	26, 1940 . . . .	99.2 ± 1.2 (5)	80.2 ± 1.53 (8)	572	19.0	19.2
	<i>November</i>					
16	26, 1940 . . . .	90.5 ± 0.42 (9)	86.1 ± 0.37 (8)	603	4.4	4.9
	<i>December</i>					
5	16, 1939 . . . .	82.75 ± 0.23 (8)	78.12 ± 0.52 (8)	257	4.63	5.6
17	26, 1940 . . . .	74.22 ± 0.14 (9)	71.55 ± 0.32 (9)	633	2.67	3.6
	26, 1940 . . . .	(Chilled 365 days)	71.8 ± 0.59 (5)	178	2.42	3.2†
	<i>January</i>					
6	29, 1940 . . . .	62.6 ± 0.5 (17)	59.07 ± 0.32 (13)	301	3.53	5.6
18	27, 1941 . . . .	56.0 ± 0.47 (8)	50.62 ± 0.28 (8)	665	5.38	9.6
	<i>February</i>					
7	16, 1940 . . . .	53.28 ± 0.37 (7)	48.75 ± 0.46 (8)	319	4.53	8.5
19	25, 1941 . . . .	46.58 ± 0.36 (12)	42.0 ± 1.0 (6)	694	4.58	9.8
	<i>March</i>					
8	20, 1940 . . . .	45.25 ± 2.58 (4)	37.5 ± 1.48 (4)	352	7.75	17.1
20	28, 1941 . . . .	43.83 ± 1.32 (6)	34.87 ± 0.57 (8)	725	8.96	20.4

† These sowings were undertaken to obtain independent index of the condition of the batch of seeds chilled for 167 days.

of two years. The other important consequence of this finding is that maximally vernalized unsplit seeds can be used to determine the degree of vernalization induced in unsplit seeds during the process of chilling—for which no other reliable index has so far been discovered. For instance, if in any simultaneous sowing of maximally vernalized unsplit seeds together with samples of any other batch or batches of seeds then in process of chilling, the observed vegetative periods of the plants are found to be similar, then the seeds tested may be considered maximally vernalized. If, on the other hand, the vegetative periods of the sample seeds are longer, then the chilling process should be continued till in later similar sowings the seeds produce plants with similar vegetative periods.

The purpose of the above experiment was to explore the possibility of maintaining the induced vernalization in unsplit seeds unimpaired for the longest possible period, and therefore the seeds were stored, as already stated, in the chilling-cabinet. This extra precaution of cold storage has since been found unnecessary. For it has been found that vernalized unsplit mustard seeds can be subjected before sowing to high temperature, without any devernalization. In a sowing of April 24, 1940, V-seeds kept throughout in cold storage 384 days produced plants with a vegetative period of  $30.53 \pm 0.41$  days (mean of 15 plants), while seeds from the same sample which were subjected to  $30^{\circ}$ — $2^{\circ}\text{C}$ . for 39 days before sowing produced plants with similar vegetative period of  $30.3 \pm 0.58$  days (mean of 13 plants). A similar sowing undertaken on August 13, 1941, from a sample (L) of a batch of seeds chilled for 167 days and kept in cold storage for 863 days and from another sample (H) which, after being kept in cold storage for 347 days, was kept at room temperature for 516 days. The observed vegetative periods were  $33.0 \pm 0.88$  days (mean of 10 plants) for L-plants and  $31.14 \pm 0.67$  days (mean of 7 plants) for H-plants. The difference of 1.86 days is not statistically significant. This indicates that storage at room temperature for over 73 weeks does not induce any devernalization.

#### *Temperature and photoperiod requirement of second phase of development*

It has been shown by Gilbert [1926], Purvis [1934], Steinberg and Garner [1936] and others that the factor of temperature must be taken into careful consideration in all photoperiodic studies. Lacking facilities for automatic control and maintenance of different combinations of temperature and photoperiod, we have utilized the natural variations in the seasonal complements of temperature and day-length to observe the effect of after-sowing environmental factors. Since the seasonal temperature and day-length vary in a similar way, i.e. high temperature is associated with long days and low temperature with short days, the data obtained from different seasonal sowings give the resultant effect of these factors varying in a similar way. Therefore, to determine the optimum after-sowing temperature and photoperiod for mustard it was necessary to observe the vegetative periods of plants grown either under (i) similar temperature ranges, or (ii) similar photoperiods throughout the year. The second alternative was adopted for the following series of observations, since the arrangements for subjecting potted plants to similar effective photoperiods throughout can be easily devised.

Tincker [1925] found that an intensity of 5 foot-candles of visible radiation is adequate for prolonging the day-length for photoperiodic reactions

In China aster Withrow and Benedict [1936] observed definite photoperiodic effect, when the intensity of the supplementary light was 0.3 foot candle and as low as 0.1 foot candle. The same authors observed that the orange and the red end of the spectrum caused the most marked photoperiodic response. Therefore, to supplement the seasonal day-length for increased photoperiod, a hanging Petromax kerosene lamp (500 c.p.) of the type commonly used as a street light, suspended from the ceiling of an open verandah, was adopted as a convenient arrangement. Except for the winter months, it was found necessary to protect the seedlings against the insects—which the bright light invariably attracted—by a mosquito curtain hung from a fine wire-net frame 4ft.  $\times$  4ft. attached to the enamel reflector of the hanging lamp. Despite the removal of all obstructions against free circulation of air in the verandah, the temperature rise from 2° to 5°C. under the lamp could not be overcome in this arrangement. For subjecting potted plants to photoperiods shorter than the seasonal day-length, the required number of hours of the morning light was cut off by keeping the pots in a well-ventilated dark chamber constructed in the glass-house.

## EXPERIMENT 12

For this experiment 10 different sowings from April, 1940, to March, 1941, were undertaken. Control and maximally vernalized unsplit seeds were sown in the two halves of several pots used for each sowing. Different sets of pots were subjected to different photoperiods. At the beginning of the light treatment, four plants were kept in each pot—two from control and two from vernalized seeds. Towards the end of the experiment, some of the plants died, and therefore in later sowings of this series the original number of plants was increased either by increasing the number of pots for each light treatment from three to four or, when the available bench space in the glass-house was inadequate, by increasing the number of plants from four to six in each pot.

The pots containing the seedlings which were subjected to supplementary artificial light were daily removed after sunset to the open verandah and were kept on a wooden platform under the hanging Petromax lantern for the required periods, after which they were brought back to the open benches of the glass-house. For photoperiods shorter than day-length, sets of pots were removed from the open benches in the glass-house to the dark chamber after sunset and were kept there till the required time in the morning, after which they were placed on the open benches in the glass-house. A control batch of seedlings, kept throughout the experiment on the open benches, was subjected to the normal day-length of the season. The position of the pots was changed every few days to secure as far as possible similar light conditions. Since all seeds were sown on the same date, it is assumed that plants in each series were subjected to a similar seasonal temperature range. The temperature variation during the light treatment did not, as will be seen later, produce any appreciable complication.

The results obtained from this series of sowings are given in Table XIV. In the first sowing of April 2, 1940, plants were subjected to photoperiods of normal day-length of 13 hours, day-length plus artificial light for three hours (16 hours), and day-length diminished by three hours (10 hours). The

light treatment began on April 9, and after three weeks' treatment, flower-buds were distinctly visible on all plants from vernalized seeds subjected to photoperiods of 13 hours and 16 hours, and the average period for the opening of the first flower in all these plants was very similar, being 34.18 days and 34.8 days, respectively. Therefore it was tentatively assumed that for plants from vernalized seeds of mustard Type 27 a photoperiodic treatment of 13 hours for three weeks was not below the optimum, and in the second sowing when the normal day-length was above 13 hours no supplementary artificial light was used. The assumption that photoperiods longer than 13 hours do not induce any further shortening of the vegetative period was verified from the subsequent sowings Nos. 3, 7 and 8. In all sowings the period of light treatment was similar, i.e. three weeks only.

TABLE XIV

*Vegetative periods of C- and V-plants under different temperatures and photoperiods*

(N, Normal day-length ; S, date of sowing ; L, light treatment)

No.	Date	Photo-period			
		(1)	(2)	(3)	(4)
1940					
1	S 2/4	16 hours	13 hours N	10 hours	
	L 9/4	C—39.8±0.66 (5) V—34.8±1.04 (5)	C—42.4±0.82 (5) V—34.2±0.65 (6)	C—52.2±1.36 (3) V—41.0±0 (3)	.. ..
2	S 7/6	14 hours N		11 hours	8 hours
	L 11/6	C—48.7±0.42 (11) V—32.4±0.7 (12)	.. ..	55.2±0.35 (9) 40.8±0.89 (11)	62.4±0.67 (7) 50.1±1.16 (8)
3	S 12/8	14 hours	13 hours N	10 hours	7 hours
	L 17/8	C—53.0±1.27 (8) V—31.8±0.71 (7)	52.6±1.28 (8) 32.1±0.76 (6)	63.9±1.9 (7) 39.5±1.34 (4)	68.0±1.0 (4) 51.6±1.32 (6)
4	S 26/9	13.5 hours		11.5 hours N	9.5 hours
	L 1/10	C—55.8±1.46 (11) V—37.1±0.82 (9)	.. ..	60.7±1.11 (13) 37.6±0.58 (11)	73.4±1.83 (11) 47.3±1.74 (10)
5	S 26/10		13 hours	10.75 hours N	9.25 hours
	L 3/11	..... .....	C—97.6±1.45 (5) V—71.4±1.07 (8)	99.2±1.3 (5) 80.0±1.53 (8)	106.4±1.53 (9) 85.0±1.11 (9)
6	S 26/11	15 hours		10.25 hours N	9.25 hours
	L 6/12	C—89.8±0.3 (9) V—84.0±0.25 (8)	.. ..	90.5±0.42 (9) 86.1±0.37 (8)	91.0±0.31 (8) 86.2±0.36 (9)
7	S 26/12	15.25 hours	13.25 hours	10.75 hours N	9.25 hours
	L 15/1/1941	C—72.7±0.46 (8) V—69.2±0.44 (9)	73.1±0.33 (9) 70.5±0.5 (8)	74.2±0.14 (9) 71.5±0.32 (9)	74.2±0.62 (9) 70.3±0.31 (9)
1941					
8	S 27/1	15.25 hours	13.25 hours	11.25 hours N	9.25 hours
	L 8/2	C—53.3±0.27 (9) V—49.2±0.24 (9)	53.8±0.3 (9) 49.9±0.29 (9)	56.0±0.47 (8) 50.6±0.28 (8)	57.8±0.63 (9) 51.9±0.73 (7)
9	S 25/2	15.25 hours		12 hours N	8.5 hours
	L 9/3	C—42.0±0.35 (9) V—37.5±0.28 (13)	.. ..	46.6±0.36 (12) 42.0±1.0 (6)	64.9±1.02 (7) 44.0±0.76 (7)
10	S 28/3	15.5 hours		12.5 hours N	9.5 hours
	L 6/4	C—40.9±0.59 (8) V—34.0±0.54 (8)	.. ..	43.8±1.32 (6) 34.9±0.57 (8)	53.6±1.7 (5) 40.4±1.16 (7)

Obviously from the nature of the data, conclusions of only a qualitative nature are justified, since of the several factors involved in these experiments, only the nature of the seeds used and actual periods for which the day-length of the season were supplemented or diminished are known. It was not possible to obtain accurate data of even the effective day-lengths which were supplemented or diminished. With regard to the temperature, only the maximum day temperature and the minimum night temperature of the glass-house were recorded, and from these no idea could be obtained as to the actual duration of the different temperatures to which the plants were subjected throughout the 24-hour period. Neither was it possible to obtain a record of humidity variation of the glass-house, beyond the afternoon records of the readings of the dry-and-wet-bulb thermometer.

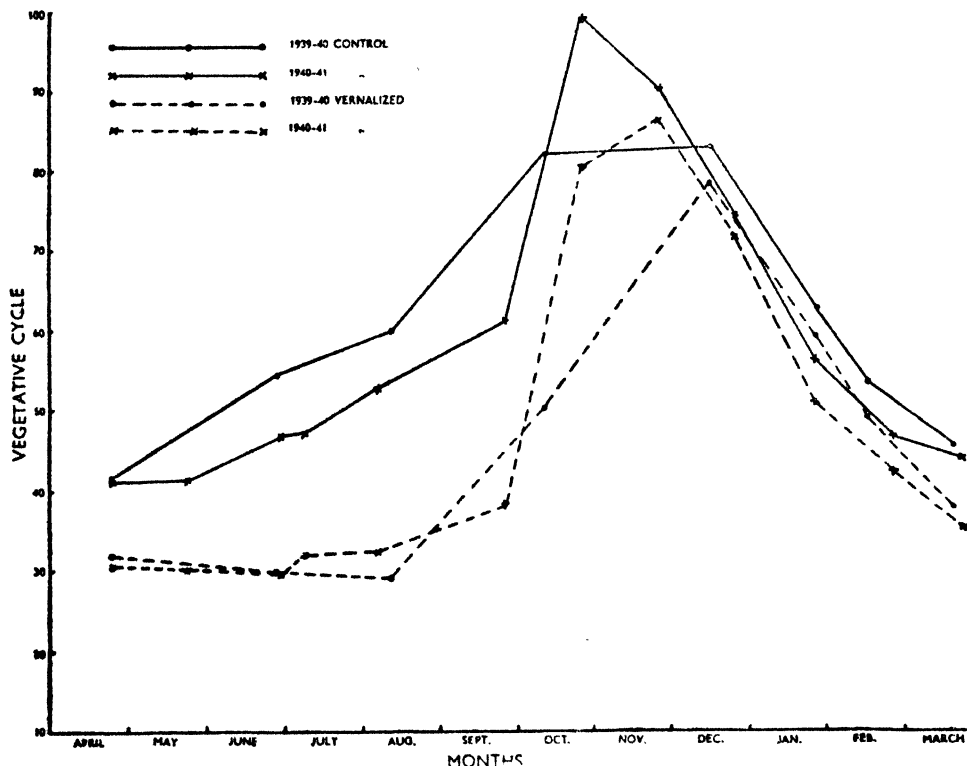


FIG. 1. Vegetative periods (days) of plants from the same batches of control and maximally vernalized unsplit seeds (mustard Type 27), in different seasonal sowings of 1939-40 and 1940-41

Despite these limitations, the following conclusions seem to be justified: (1) Under all similar temperatures and photoperiods so far studied plants from vernalized seeds of mustard Type 27 flower significantly earlier than those from the untreated controls (Figs. 2 and 3). (2) No critical photoperiod is discoverable for mustard Type 27, since flowers are produced from plants which have been subjected to a photoperiod of 16 hours in April sowing (treatment 1) as well as 9½ hours for the first three weeks in October sowing (treatment 4) and afterwards from November 25, 1940, to January 19,

1941, to normal day-lengths of  $10\frac{1}{2}$ - $10\frac{1}{4}$ - $10\frac{1}{2}$  hours. But under all temperature ranges prevailing in Almora, except the limiting one during sowing VI,

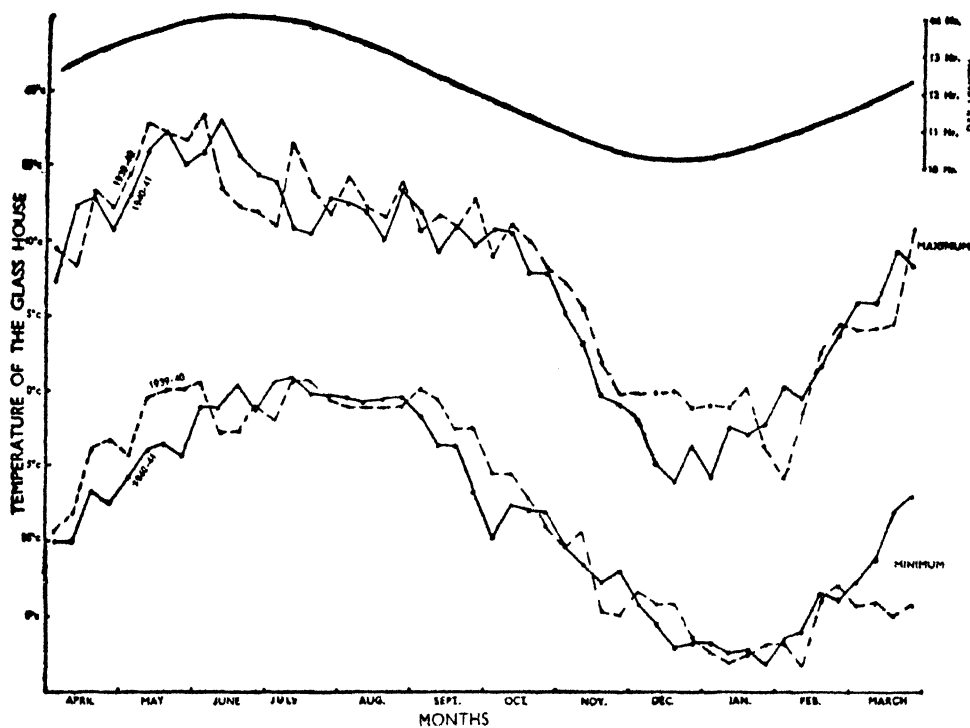


FIG. 2. Weekly average maximum and minimum temperatures of the glass-house (1939-40 and 1940-41) and the day-length of Almora

of November 26, 1940, the observed vegetative period progressively diminished as the photoperiod was increased up to 13 hours. Increase in photoperiod beyond 13 hours—16 hours in April, 14 hours in August, 15 hours in December,  $15\frac{1}{2}$  hours in January and February—did not produce any further shortening of the vegetative period. From this it can be concluded that the optimum photoperiod for the second phase of development of mustard Type 27 is not more than 13 hours. Incidentally it is shown that a temperature rise of  $2^{\circ}$ - $5^{\circ}\text{C}$ . during supplementary light treatment for two to three hours produces no significant difference as far as the vegetative period is concerned. (3) The increased vegetative periods observed in winter sowings are due mainly to the prevailing low temperature and not to diminished day-length. For it will be seen that in sowing 1 (when the average maximum day temperature was  $31^{\circ}\text{C}$ .) the observed vegetative periods with photoperiods of 13 hours for three weeks were 34.18 days for V-plants and 42.4 days for C-plants, but in November sowing (No. 6) the vegetative periods observed with a photoperiod of 15 hours for three weeks (when the average maximum day temperature varied from  $20^{\circ}$  to  $15^{\circ}\text{C}$ .) were 84 days for V-plants and 89.8 days for C-plants. (4) The optimum day-temperature of the second phase of development of mustard Type 27 appears to be  $30^{\circ}\text{C}$ ., for under all similar photoperiods in all sowings from April 24 to August 12, both in 1939 and 1940,

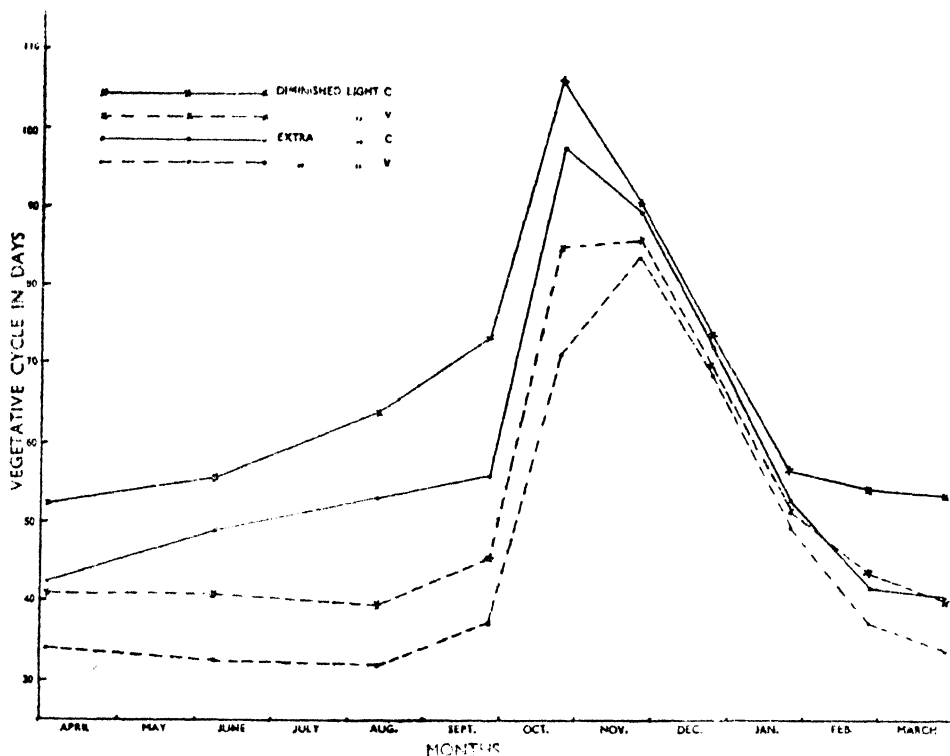


FIG. 3. Vegetative periods of plants from the same batches of control and maximally vernalized unsplit seeds (mustard Type 27) under photoperiods longer and shorter than seasonal day-length

(Tables XIII and XIV) when the maximum temperature varied from  $30^{\circ}$  to  $38^{\circ}\text{C}.$ , the observed vegetative periods of V-plants were similar and minimum. When the maximum day temperature was below  $30^{\circ}\text{C}.$ , however, the vegetative periods were found to increase progressively. (5) The differential response of C- and V-plants to similar after-sowing environmental factors observed in seasonal sowings of 1939-40 and 1940-41 can be explained if the minimum night temperature is taken into consideration. In sowings of June, July and August (Tables XIII and XIV), when the natural seasonal complements of day temperature and day-length were optimum (Fig. 2), the vegetative period of V-plants were remarkably similar, while those of C-plants were found to increase steadily from June sowings onwards. From the minimum temperature curve of the glass-house, it will be seen that from mid-April to the first week of June, the night temperature is lower than from the last week of June to the end of August. Furthermore, the period from April till the monsoon starts—about the middle of June—is the driest one in Almora, and the temperature of the moist soil in pots is generally  $3^{\circ}\text{--}5^{\circ}\text{C}.$  lower than the recorded air temperature of the glass-house. The average minimum night temperature of the air for five days following April 24, 1940, was  $13^{\circ}\text{C}.$ , and for the five days following May 22, it was  $16.5^{\circ}\text{C}.$ , while the minimum night temperature for five days following June 7, 1940, was  $20^{\circ}\text{C}.$  The prevailing night soil temperature in Almora from late April

to the beginning of June is thus of the order of the temperature required or vernalization (Experiment 2), and it is reasonable to suppose that in this region in sowings of April and May partial natural chilling takes place in the case of untreated controls, at least to a greater extent than during the hotter nights of June, July and August. It was shown in our preliminary report [Sen and Chakravarti, 1938] that sprouted mustard seeds of Type 27 chilled for only four days, produced plants in the September sowing of 1937 which flowered nine days earlier than those from the control sprouted seeds. In the case of plants from maximally vernalized seeds, however, neither the cooler nights of April and May, nor the hotter nights from June to August, affect the vegetative period. The verification of the above assumption is seen in the minimum difference in the vegetative periods of C- and V-plants, in sowings of November and December, where the advantage of the V-plants (pre-supply of low temperature) is reduced to a minimum by the natural winter temperature of this region.

#### DISCUSSION AND CONCLUSIONS

From the results of the experiments described, it is evident that all the five strains of mustard—Type 27, C 11, C 9, yellow *sarson*, and *raya* O.B/I—respond to vernalization, and chilled unsplit seeds of mustard show all the characteristics of vernalized seeds. For instance, in the case of mustard Type 27, which has been used for most of the experiments, unsplit chilled seeds produce plants which flower earlier than plants from untreated seeds, the vernalization induced in unsplit chilled seeds increases with increased dose of chilling until, under optimum conditions, maximum vernalization is attained by chilling for six weeks. Prolonged chilling up to 365 days neither induces any higher degree of vernalization in unsplit seeds nor any devernalization. These observations are in accord with the findings of Lojkin [1936] in connection with vernalization of winter wheat. The same author observed that under field conditions the percentage of germination of vernalized wheat was lower than for untreated seeds, and this has also been observed by us (unpublished data) in the case of vernalized winter wheat. But in the case of vernalized unsplit mustard seeds, the germination has been found to be similar to that of control seeds [Sen and Chakravarti, 1938]. From Tables IX, X and XI, in which are given the data obtained from normal seasonal sowing under field conditions, it will be seen that the earliness in flowering of plants from maximally vernalized mustard seeds was 17.76 days for C 11, 25.4 days for Type 27 and 29.57 days for C 9. In the case of mustard Type 27, plants from vernalized seeds have been found to flower earlier under all combinations of after-sowing temperature ranges and photoperiods studied (Tables XIII and XIV). Thus, a definitely earlier harvest can be insured by the use of vernalized unsplit seeds of mustard, which also have the advantage that they are capable of being stored for over two years without deterioration.

#### *Yield*

For practical agriculture, the yield and the quality of the crop are as important as earlier harvest, if not more important. Whether earlier harvest obtained from vernalized mustard seeds can be associated with higher yield and better quality of crop, under otherwise similar cultural conditions, depends

on the environmental factors prevailing during the period of seed-setting and seed-ripening. In the case of wheat, Kirichenko [1934] observed that, lacking the photoperiodic requirement of the third phase, seeds would not set, as the pollen became sterile. Ali Mohammad and Ahmad [1940] have shown that the oil content of mustard depends, among other factors, on the temperature during seed formation. In the case of an insect-pollinated crop, the population of the pollinating insects during the period of full bloom is also an important factor which determines the yield. Furthermore, it should be realized that extreme shortening of the life-cycle under optimum environmental conditions would in all probability produce ephemeral plants and obviously their yield would be considerably lower than normal. For instance, in small field-plot sowings of June, 1938, the total period from sowing to harvesting of mustard Type 27 was 99 days for V-plants, and the yield was  $4.52 \pm 0.48$  grams (mean of 38 plants) per plant. In sowings of October, 1939, when the V-plants took 208 days to complete the life-cycle, the average yield per plant was  $22.9 \pm 1.45$  grams (mean of 91 plants).

To derive the maximum advantage from vernalized seeds, new optimal sowing dates should be discovered for different regions, as Whyte [1939] has already pointed out. Since the environmental requirements of the different phases of development of a crop are not identical, and yield and quality of the crop are the final expression of the life-cycle, higher yield and better quality of crop can be expected from vernalized seeds if the facility offered by early flowering can be utilized to secure (preferentially) for V-plants better environmental factors for the completion of the life-cycle. Greater yield can also be expected if, by earlier harvest, the hazards of pests, drought, excessive rain, frost or snowfall can be avoided, or at least partially mitigated. Two cases may be cited, one of mitigation of caterpillar injury, the other, of greater damage resulting from snowfall, observed by us in connection with V-plants. In our first outdoor sowing of June 4, 1938 (Experiment 2) yield per plant was recorded. The average yield per V-plant was 4.55 grams (mean of 38 plants) and per C-plant, 1.98 grams (average of 25 plants), but this difference was not statistically significant; the higher average of V-plants was in reality due to the differential damage caused by caterpillar attack. The less advanced C-plants with their softer tissue system were more severely damaged than the taller V-plants, and some of the former were completely destroyed. In a small field-plot sowing of October 6, 1939, with six replications each of control and vernalized seeds, the yield per plant was again recorded. The yield observed per V-plant was 22.9 grams (mean of 91 plants) and 27.94 grams (mean of 95 plants) for C-plants. In this case also, though the observed difference was not statistically significant, the variation can be explained by the fact that on February 6 and 10, 1940, during the full-bloom period of the V-plants, which flowered 28.89 days earlier than the C-plants, two heavy snowfalls occurred, causing greater damage to the V-plants than to the less advanced control plants. But in our preliminary observations with pot-culture plants grown in the protected environment of a glass-house, the yield observed per V-plant has been found to be greater than that of C-plants, and this alike in off-season sowings of July and seasonal sowings of October. In sowings of July 11, 1938, where seeds chilled for different periods were used, the records of the following four characters of each plant were

taken : (i) time of opening of flowers (Table II) ; (ii) time of completion of flowering ; (iii) time of maturity ; (iv) yield per plant. The results of the statistical analysis of the data submitted to Prof. P. C. Mahalanobis of the Statistical Institute, Calcutta, show that in all these four characters V-plants had the advantage, i.e. compared with C-plants, V-plants flowered earlier, flowering was completed earlier, seeds matured earlier and their weight per plant was greater. Thus, for example, the mean yield per C-plant was 0.499 gram and per V-plant (seeds chilled for six weeks) 1.025 grams, a difference which is statistically significant at 1 per cent level. In sowings of October 18, 1938, the observed yield (mean of 11 plants) per C-plant was 0.96 gram, while the yield (mean of eight plants) per V-plant was 1.68 grams, a difference significant at 5 per cent level. But from these data no definite conclusion is justified regarding yield of V-plants under field conditions. Therefore, pending the results of experiments now in progress to determine the optimum environmental requirements of the third phase and optimum temperature for seed-ripening, systematic investigation regarding the possibility of associating earlier harvest with higher yield and better quality of crop from vernalized seeds has been postponed.

With reference to the effect of drying and storage of vernalized seeds, Lojkin [1936] found that vernalized seeds of winter wheat when air-dried for four weeks at 1° and 15°C. are partially or completely devernalized. Gregory and Purvis [1938] found that when maximally vernalized seeds of winter rye are dried, the process of devernalization sets in after six weeks, and in course of 20 weeks complete devernalization takes place. Purvis and Gregory [1937] in explaining this devernalization by drying in terms of a suggested scheme of vernalization maintain that drying induces a reversal of the reaction which produces the substance responsible for early flowering in plants from vernalized embryos. But it will be seen from Experiment 11 that vernalized unsplit seeds of mustard can be dried for a period of 863 days without any observable devernalization. Therefore it can be concluded that drying even at room temperature does not devernalyze unsplit vernalized seeds of mustard. The contradictory effects of drying on vernalized seeds of mustard and of wheat and rye may be due either to the difference in the nature of the embryos concerned, or to the different stages of growth of the embryo of the vernalized seeds of mustard and of wheat and rye. The embryos of the vernalized seeds of wheat and rye developed to the seedling stage, while obviously in the case of vernalized unsplit seeds of mustard the growth of the embryo is confined within the elastic limit of the seed-coat. That the different effects of drying are not due to the types of the embryos concerned but to the stages of their development during the period of low-temperature treatment is suggested from the fact that in the case of rye also, when the developing embryo is chilled in the ear, no devernalization takes place when these seeds ripen and become dormant [Gregory and Purvis, 1938]. In natural vernalization of the seeds, the growth of the embryo is limited within the confines of the testa, which is also the case with chilled unsplit mustard seeds. Therefore it seems reasonable to conclude that so long as the growth of the embryo is confined within the elastic limit of the testa, it can retain the effect of chilling unimpaired for long periods. Investigations are in progress to find out the specific protective character of the seed-coat.

Gregory and Purvis [1938] have shown that the capacity of pre-chilled seeds to produce plants with shorter vegetative period is not due to delayed germination but to the specific effect of low temperature on the embryo, alike during the period of its development during seed formation and when the dormant embryo is activated. In our experiments with mustard Type 27, earliness in flowering has been observed in plants both from chilled unsplit seeds as well as from unchilled seeds which developed during the winter months (Experiment 9). This natural vernalization, at least partial, induced by the prevailing low temperature during the period of seed ripening suggests interesting possibilities for vernalization. It is a common experience in the tropics that imported seeds of some of the winter annuals from colder climates give very good results, but fail to produce seeds as good as those of the parent stock. If the cause of the seed deterioration be mainly due to prevailing high temperature during the period of seed-ripening in the tropics then this defect could be corrected by shortening the vegetative period through the use of V-seeds, provided the crop responds to vernalization.

Plants from untreated seeds of mustard Type 27 have been found to flower under all the different seasonal complements of temperature and day-length prevailing in Almora. In our glass-house the maximum day temperature during the year varies from  $40^{\circ}$  to  $10^{\circ}\text{C}$ . and the minimum night temperature from  $22^{\circ}$  to  $1^{\circ}\text{C}$ . The day-lengths vary from 10.2 hours to 14 hours. In all sowings, under similar environmental conditions, V-plants flower significantly earlier than C-plants, yet it would appear that low temperature is not an obligatory factor for inflorescence of mustard Type 27. For it will be seen from Table XIII that, when the minimum night temperature averaged  $20^{\circ}\text{C}$ . (Fig. 2) during the months of June and July, 1940, plants from untreated seeds flowered in 46.75 and 47.18 days, respectively (Nos. 11 and 12), which was only about half the periods required for flowering by similar plants in winter sowing of October and November, namely, 99.2 and 90.5 days, respectively (Nos. 16 and 17). That the shorter vegetative period observed in summer is not due primarily to optimum photoperiod, but to temperature, will be seen from the results of sowing No. 1 (Table XIV), where the observed vegetative period of C-plants grown under 10 hours photoperiod (shorter than Almora winter day-length) for three weeks and subsequently under normal day-length of 13 hours was 52.2 days, while in sowing No. 3 under exactly similar photoperiods the observed vegetative period was 69.9 days. Therefore it can be concluded that for mustard Type 27, in spite of the fact that there is no specific low-temperature requirement of the first phase, low temperature, whether pre-supplied to the embryo during seed formation or to the embryo of the unsplit seeds or of sprouted seeds, will shorten the vegetative period of the plants. The quantitative nature of the effect of low temperature on the development of mustard is proved by the fact that the vernalization induced increases up to a maximum with increased dose of chilling (Experiment 2).

In the case of winter rye, Purvis and Gregory [1937] found that seedlings subjected to decreased photoperiod for six weeks at the initial stage will advance to the reproductive stage earlier. From the data given in Table XIV, it will be seen that increased photoperiod for the first three weeks will shorten the vegetative period of mustard Type 27. In sowing 2 of June 6, 1940,

(when the day temperature was optimum and the minimum night temperature was about 20°C.), the V-plants flowered earlier than the corresponding C-plants under all the three photoperiodic treatments. As the photoperiods were shortened from the normal day-length of 14 hours, the vegetative periods of both V- and C-plants increased. The vegetative periods of plants subjected to eight hours photoperiod for the first three weeks and subsequently to normal day-length of 14 hours were 62.4 days for C-plants and 50.1 days for V-plants, while the vegetative period of C-plants subjected throughout to normal day-length of 14 hours was 48.7 days, which was similar to that of V-plants (50.1 days) subjected to a shorter photoperiod (Plate II), for the observed difference of 1.4 days is not statistically significant. The C-plants under 14 hours photoperiod were subjected to the minimum temperature which averaged 20°C. throughout their first and second phases, yet they flowered at the same time as the V-plants subjected to diminished photoperiod. Thus it can be concluded that a similar shortening of the vegetative period can be obtained either by pre-supply of low temperature to the embryo or by subjecting seedlings to increased photoperiod. From the observations recorded it would appear that the original concepts of Lysenko's theory of phasic development of annual seed crops are not applicable to mustard Type 27, either in regard to the obligatory qualitative nature of the changes produced by low temperature during the first phase, or the strict dependence of each phase on the completion of the preceding phase.

#### SUMMARY

Vernalization response of different strains of mustard—Type 27 from New Delhi, Types C 11 and C 9 from Cawnpore, *raya* O.B/1 and yellow *sarson* from Lyallpur—has been observed. Most of the environmental studies were, however, carried out with mustard Type 27. Simple techniques, without facilities of electric supply, for vernalization of seeds and determination of after-sowing optimum temperature and photoperiod have been described. From the observed vegetative periods of plants grown in pots as well as in small field plots the following conclusions have been reached :—

1. All the five strains of mustard respond to vernalization, i.e. plants from vernalized seeds flower earlier than those from untreated seeds.

2. For a similar dose of chilling the vernalization response of different strains of mustard varies.

3. Seeds which sprout as also those which remain unsplit, during the period of chilling are vernalized; but for the same dose of chilling, earliness observed in plants from sprouted chilled seeds is greater. The degree of vernalization induced increases to a maximum with increased dose of chilling. Under optimum chilling conditions maximum vernalization is induced in unsplit chilled seeds of mustard Type 27 in six weeks; further prolongation of chilling up to 365 days does not induce any higher degree of vernalization, nor any devernialization.

4. While drying is fatal for sprouted chilled seeds of mustard, chilled unsplit seeds can be dried, and drying does not affect subsequent germination.

5. The observed vegetative periods of plants (Type 27) from progenies of seeds vernalized for three successive generations do not indicate any transmission of the effect of vernalization to the offspring.

6. When growth of the embryo is confined within the elastic limit of the seed-coat, the chilled seeds can be dried and stored for long periods (863 days so far observed) without any resultant devernalization.

7. Under all similar after-sowing temperature range and photoperiods so far studied, plants from vernalized unsplit seeds flower earlier. Thus an earlier harvest can be obtained by the use of vernalized unsplit seeds. The possibilities of associating yield with earlier harvest are discussed.

8. Mustard Type 27 has no obligatory low-temperature requirement of the first phase, for plants from untreated seeds will flower even when the minimum night temperature is 20°C. or more.

9. Partial natural vernalization is induced in mustard Type 27 when the embryo develops under low temperature.

10. According to the prevalent categories, mustard Type 27 is neither a short-day nor a long-day plant, since it flowers under photoperiods of 10 hours as well as of 16 hours. But it is not indifferent to photoperiod.

11. Under all temperature ranges of the Almora climate, with an increase of photoperiod from 10 hours to 13 hours for the initial three weeks, plants from both untreated and vernalized seeds will flower significantly earlier. Photoperiods longer than 13 hours (up to 16 hours) induce similar effects in regard to the flowering date of mustard Type 27, and therefore 13 hours may be taken as the optimum photoperiod.

12. Under similar photoperiods greater shortening of the vegetative period is observed with increased temperature-range. Increased vegetative period during winter is primarily due to low temperature and not to short days. The optimum temperature for the second phase of development is 30°C.

13. Within limits, the effect of low temperature during the first phase, and of photoperiod during the second phase, in shortening the vegetative period of mustard Type 27 is of a quantitative nature.

14. Embryo of mustard subjected to low temperature, or seedlings subjected to optimum photoperiod, can produce similar shortening of vegetative period.

15. In the light of the experimental data presented, the original concepts of Lysenko's theory of phasic development of annual seed crops is not applicable to mustard Type 27, either in regard to the obligatory qualitative nature of changes produced by low temperature during the first phase, or the strict dependence of each phase on the completion of the preceding phase.

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# ENTOMOLOGICAL INVESTIGATIONS ON THE LEAF-CURL DISEASE OF TOBACCO IN NORTHERN INDIA

## V. BIOLOGY AND POPULATION OF THE WHITE-FLY VECTOR [*BEMISIA TABACI* (GEN.)] IN RELATION TO THE INCIDENCE OF THE DISEASE

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(With Plate III and five text-figures)

THE white-fly, *Bemisia tabaci*\* is a well-known pest of cotton in several parts of India and has been reported to be responsible for the periodic failure of some American varieties of this crop in the Punjab [Husain, 1933]. The white-fly, which occurs in very large numbers, damages cotton by de-sapping the leaves, which consequently get disfigured and discoloured. It is also found on tobacco almost all over India, though it is not so common in well-known tobacco-growing areas, e.g. Guntur in the Madras Presidency, in south India. This white-fly is also reported to transmit leaf-curl of cotton in the Gezira district of the Sudan [Kirkpatrick, 1931] and leaf-curl of tobacco in Southern Rhodesia [Storey, 1932]. Thung [1932] reported that this species occurs in the Vorstenland districts of Java, where it is a vector of 'Kroepoek' disease of tobacco.

In India, *Bemisia tabaci* is not known to cause any virus disease to cotton, though its incidence on this crop is generally very high. In the case of tobacco, however, we have already conclusively shown that it is a very important vector of leaf-curl disease, which is common in North and Central India, [Pruthi and Samuel, 1937, 1939]. This white-fly has a large number of other food-plants in north Bihar (Pusa), a number of which also suffer from leaf-curl diseases, and in the case of some of them the white-fly has been shown by us to act as a vector of the disease [Pruthi and Samuel, 1941].

In view of the great importance of *Bemisia tabaci* as a vector of tobacco leaf-curl virus or viruses, the writers have studied its life and seasonal histories, range of food-plants, incidence on tobacco at different times of the year, etc., during the past four or five years at Pusa, and the results of these investigations are reported in the following pages :

\* Silvestri (Entomologia Applicata, *Gli Insetti*, I, p. 401, 1934), considers *Bemisia gossypiperda* M. and L. to be a synonym of *B. tabaci* (Gennad.) (Gennadius, *Agric. ellenica*, 1889).

## FOOD-PLANTS

The occurrence of *Bemisia tabaci* on several food-plants in the Punjab has been recorded by Husain and Trehan [1933, 1936]. In a previous communication, the present writers [Pruthi and Samuel, 1939] reported several cultivated and wild plants as hosts of this white-fly at Pusa. During the past two years, we have made a thorough survey of the alternate hosts of this species in the environs of Pusa and some other localities in north Bihar, and a list of all the plant hosts so far observed is given in Appendix I. Several of these plants show symptoms of some leaf-curl disease almost similar to that in tobacco and in the case of some we have, as already stated, experimental evidence that the virus is the same which causes leaf-curl in tobacco.

## LIFE-HISTORY

Some observations on the life-history of *B. tabaci* made in cotton fields in India have been recorded by Misra and Lamba [1929], Husain and Trehan [1933], etc. Our observations differ in several important respects from those of the workers named above and are briefly described below :—

In Bihar, tobacco is usually sown in August and transplanted towards the end of September or early in October. The white-fly begins to make its appearance on this crop about the middle of October. It is generally found on the under surface of leaves, but all the leaves of a plant or all the plants are not equally infested ; in fact some are entirely free, but those leaves which are infested are generally fully covered with various stages of the fly. Therefore, in addition to causing the leaf-curl disease, the white-fly directly damages the leaves by de-sapping them and injecting their saliva therein. The honey-dew secreted by numerous nymphs is conducive to the development of sooty moulds on the leaves.

*Copulation*

The most active period of breeding is early autumn (September-October) and spring (February-March). Copulation occurs 2-6 days after emergence. An infested leaf, when closely examined, reveals a number of white-fly adults, sitting in groups of two or three in close contact with one another, and almost simultaneously shaking their wings preparatory to copulation. One male and one female are thus often seen together, but sometimes there are two males one on each side of a single female. The male as a rule dies within 24 hours after copulation, but the female after this process moves about restlessly for some time on the leaf surface, apparently in search of a suitable place for oviposition. By the time it actually deposits eggs, it acquires a full coating of powdery meal on its wings. The period between copulation and oviposition varies from one to two days in April-May and two to four days in October-December.

*Oviposition*

As the time for oviposition draws near, the female starts spinning on the under surface of the leaf, minute, irregular or circular patches made up of

network of thin, white fibres, composed of mealy-powder derived from its wings, which it scrapes off with its antennae and legs. The ovipositing female, therefore, generally looks somewhat discoloured, owing to the absence or disarrangement of its powdery stuff. After spinning for about 20 minutes, it deposits the first egg and covers it with a few powdery strands, and then lays another egg in close contact with the previous one and similarly covers it. Sometimes eggs are also laid almost entirely exposed. While laying eggs, the female raises the glandular hairs present on the surface to an upright position and clothes them densely with powdery meal. These hairs probably afford protection to the eggs against enemies.

Oviposition records were taken every month during the three tobacco seasons of 1936-39 (Table I). For this purpose, freshly emerged white-flies were taken and each pair was put in a micro-cage described by us in previous communications [1939, 1941]. The cage was fixed on the lower surface of a leaf of a young potted plant enclosed in a glass chimney. After twenty-four hours, the tube containing the pair was removed and adjusted on a fresh spot on the same leaf. This was repeated every day and eggs deposited on various spots were recorded.

The maximum number of eggs laid by a single female in captivity was 77 in 1936 (October), 69 in 1937 (September), 168 in 1938 (April) and 206 in 1939 (March). The maximum oviposition period varied from 9 to 12 days. The average number of eggs laid by a single female was 44 in 1937 and 77 in 1938. The female was found capable of laying up to a maximum of 56 eggs in twenty-four hours, and the egg-laying was distributed throughout the period. The highest number of eggs deposited by a female on cotton plants, according to Husain and Trehan [1933] was 119 in 18 days, the average being 28 in 1929 and 43 in 1930.

The meteorological conditions of the period during which the above observations were taken are summarized in Appendix II. An examination of the Appendix and Table I (containing the oviposition records) will show that the capacity for egg-laying is largely governed by the prevailing temperature and humidity conditions. As the temperature goes up, as is the case from March to May, the number of eggs laid per day increases, but the aggregate number remains almost the same. Under the opposite conditions (in December and January), the number of eggs deposited is considerably reduced and the oviposition period is prolonged.

#### *Duration of immature stages*

Husain and Trehan [1933] described the various stages of the white-fly, but their illustrations are not satisfactory. Therefore, drawings of the immature stages are included in this paper and the durations of various instars are briefly described below :—

*The egg* (Plate III, figs. 1-2).—The incubation period of the egg was 3-4 days in April-July, 3-10 days in August-March, the longest period observed being 7-10 days in December. The incubation period on cotton recorded in the Punjab was 3-33 days. The eggs are often preyed upon by a mite abundant on tobacco during August to October. They are also affected by intense cold, with the result that their hatching is indefinitely delayed.

TABLE I

*Records of oviposition of Bemisia tabaci during 1936-39*

Date of emergence	Date of beginning of oviposition	Total number of eggs laid and the number of days during which they were laid
<b>1936—</b>		
30 August . . . .	1 September . . . .	51 (4)
23 September . . . .	25 September . . . .	62 (4)
17 October . . . .	23 October . . . .	77 (4)
21 November . . . .	6 December . . . .	30 (6)
<b>1937—</b>		
7 January . . . .	14 January . . . .	36 (7)
17 February . . . .	21 February . . . .	47 (5)
25 March . . . .	30 March . . . .	55 (5)
20 April . . . .	23 April . . . .	39 (4)
17 May . . . .	19 May . . . .	58 (4)
14 June . . . .	18 June . . . .	35 (4)
9 July . . . .	12 July . . . .	31 (3)
6 August . . . .	8 August . . . .	27 (3)
1 September . . . .	4 September . . . .	69 (5)
2 October . . . .	4 October . . . .	50 (6)
2 November . . . .	5 November . . . .	42 (8)
6 December . . . .	11 December . . . .	35 (9)
<b>1938—</b>		
16 January . . . .	20 January . . . .	39 (12)
21 February . . . .	26 February . . . .	44 (5)
28 March . . . .	30 March . . . .	58 (5)

TABLE I—*contd.*

Date of emergence	Date of beginning of oviposition	Total number of eggs laid and the number of days during which they were laid
1938— <i>contd.</i>		
24 April . . . .	26 April . . . .	168 (5)
5 May . . . .	7 May . . . .	163 (5)
17 May . . . .	19 May . . . .	149 (5)
25 May . . . .	29 May . . . .	140 (5)
12 June . . . .	15 June . . . .	39 (4)
19 June . . . .	22 June . . . .	42 (3)
6 July . . . .	8 July . . . .	48 (4)
12 July . . . .	16 July . . . .	32 (3)
6 August . . . .	8 August . . . .	48 (4)
24 August . . . .	26 August . . . .	55 (4)
10 September . . . .	14 September . . . .	120 (7)
3 October . . . .	8 October . . . .	79 (6)
4 November . . . .	10 November . . . .	41 (5)
10 December . . . .	15 December . . . .	37 (9)
1939—		
25 January . . . .	30 January . . . .	33 (10)
20 March . . . .	23 March . . . .	206 (5)
16 April . . . .	20 April . . . .	131 (4)

Meteorological data for the above periods are given in Appendix II.

*First instar nymphs* (Plate III, fig. 3).—The young nymph casts its first moult in 3-5 days in August-September, 8-10 days in December-January and 3-5 days in May-June. The average duration of the first instar was 6 days. A portion of the cast skin is often seen adhering to the caudal end of the nymph but within a few minutes it dries up and gets blown off by wind.

*Second instar nymph* (Plate III, fig. 4).—The nymph moults in 2-6 days in August-September, 6-9 days in December-January and 1-4 days in April-May.

*Third instar nymph*.—The nymph moults in 2-7 days in August-September, 6-9 days in December-January and 2-4 days in April-May.

*Pupa* (Plate III, fig. 5).—The pupal period occupies 2-5 days in August-November, 4-6 days in December and 3-6 days in January. A freshly-formed pupa is generally thin and flat, sub-elliptical, light-yellow, but soon becomes convex and yellow, with the margin broadly crenulate.

The legs which are well developed in the first instar begin to degenerate in the succeeding instars, and are replaced by stump-like suckers in the second and third instars, while they are curved and unsegmented in the pupa. The dorsal spines, on the other hand, which are practically absent in the first instar gradually make their appearance in the subsequent instars, i.e. 3 pairs in the second and third instars, and seven pairs in the pupa. The number and arrangement of the dorsal spines of the pupa bred on cotton described by Husain and Trehan [1933] is also constant in the pupae bred from tobacco. But so far, we have not met with any case of the total absence of the dorsal spines mentioned by those authors.

The average duration of each of the three nymphal instars mentioned above is 4-5 days. The total period of the three instars was 12-21 days in August-March, 10-14 days in April-July and 18-24 days in October-December.

*Duration of the life-cycle*.—The life-cycle of the white-fly as observed by Husain and Trehan [1933] was 14-21 days during April to September, the longest being 107 days. According to our observations the life-cycle lasted 17-32 days during August-March in 1937-38 and 16-39 days in 1938-39. The longest life-cycle noticed was 39 days in December and the shortest 11 days in April.

In the laboratory, the white-fly completed twelve broods in the course of one year.

*Emergence of adults*.—The newly emerged insect often remains in contact with its empty pupal case for about 25 minutes, by which time the wings dry and unfold themselves to assume their normal shape and size. Freshly emerged adults have at first semi-transparent wings which in a day or two get covered with white mealy powder. Although emergence of adults generally takes place during the day, sometimes it also takes place at night.

Frequent collections of adults made from large rearing cages at different times of the year, have shown that the proportion of males to females is high during March-August, while it is low during September-February. The number of females emerging from the pupae is remarkably larger than that of males during winter months, viz. November-January.

*The adults*.—The abdomen of the male is creamy-yellow and tapering posteriorly; in the female, it is slightly bigger and broader and is distinctly yellow (Plate III, fig. 6). Both male and female prefer cool and shady places for breeding purposes. In summer, they hide in the day to avoid strong sun-light, and are active in the mornings and evenings. In winter, they are observed in the field between 8 A.M. and 12 noon and 2-30 and 5-30 P.M. While flying from one field to another they often exhibit whirling movements in the air, but when moving from one plant to another they invariably jump;

*Bemisia tabaci* ( Gen. )

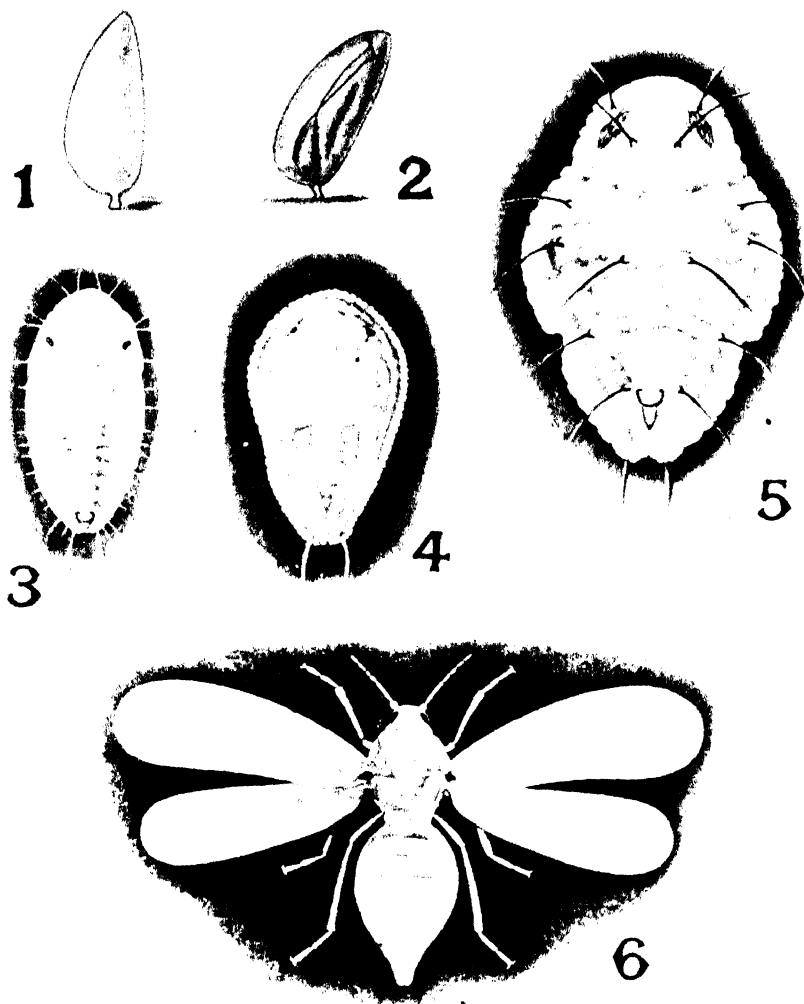


FIG. 1. A freshly laid egg ( $\times 135$ )

FIG. 2. The egg-shell after hatching of the young nymph ( $\times 135$ )

Note the longitudinal slit along one side

FIG. 3. Nymph, first instar ( $\times 135$ )

FIG. 4. Nymph, second instar ( $\times 90$ )

FIG. 5. Pupa ( $\times 80$ ) Note the presence of eight pairs of spines (seven dorsal and one anal) and their arrangement.

FIG. 6. Adult white-fly, female ( $\times 45$ )



sometimes a jump may be as long as 20 feet or even more. Tobacco plants kept in pots at a height of 30 feet from the ground level, became infested with white-flies, showing that they are capable of flying up to that height.

*Longevity of the two sexes.*—The duration of life was found to vary with the sexes. Males were usually short-lived, their average life in April-August being 4 days, and that of females 8 days. The average life in winter (November-January) for the two sexes was 7 and 12 days respectively. Copulation was also observed to influence the duration of life. In the case of males, the average duration in March-May before copulation was 4·5 days, and in November-January 6·5 days, while the corresponding figures for the two seasons after copulation were 3·8 and 5·5 days respectively. In the case of females, the average duration of life in March-May before copulation was 6 days, and in November-January was 8·5 days; the corresponding figures after copulation were 7·5 and 12·5 days respectively. These observations show that the length of life in males is shortened as a result of copulation, while in females it is increased.

When starved, the two sexes lived on an average for 1·5 and 2·5 days during March-May and November-January respectively.

#### *Sexual dimorphism*

There is sexual dimorphism among the adults and pupae of the white-fly and the two sexes can be recognized easily. The adult female differ from the male by having a comparatively stouter abdomen and longer wings, and its pupa is bigger in size than that of the male.

#### *Parthenogenesis*

The phenomenon of parthenogenesis mentioned by Husain and Trehan [1933] has also been observed by us. At Pusa it was noted both in the spring and autumn. Freshly emerged females were kept isolated in micro-cages, allowing them no chances for sexual reproduction. One to two weeks later, 12-37 eggs were laid per female, and the progeny arising from them consisted of only males, which were smaller in size than those produced normally.

### THE POPULATION OF THE WHITE-FLY ON TOBACCO AND SOME OTHER HOSTS AT DIFFERENT TIMES OF THE SEASON

We have already stated that there is a large number of wild and cultivated plants which act as hosts for the white-fly. Sunn-hemp, which is one of its important food-plants, is sown at Pusa about the middle of May. The white-fly appears on it in June, when the plants are about two weeks old. On *urid*, *patwa* and *arhar* it appears early in August having migrated to them from *duranta* and several other weeds, e.g. *Solanum nigrum*, *Vernonia cinerea*, *Euphorbia hirta*, *Ageratum conyzoides*, *Anisomeles orata*, *Launea asplenifolia*, *Scoparia dulcis*, etc. The white-fly multiplies rapidly on the above mentioned plants up to September, then there is a gradual decline in its numbers and about the middle of November there is practically no white-fly on these plants. Sunn-hemp remains in the field up to the end of September for manurial purposes and up to the end of January for seed purposes. *Patwa* lasts up to the end of January, but *arhar* remains till March.

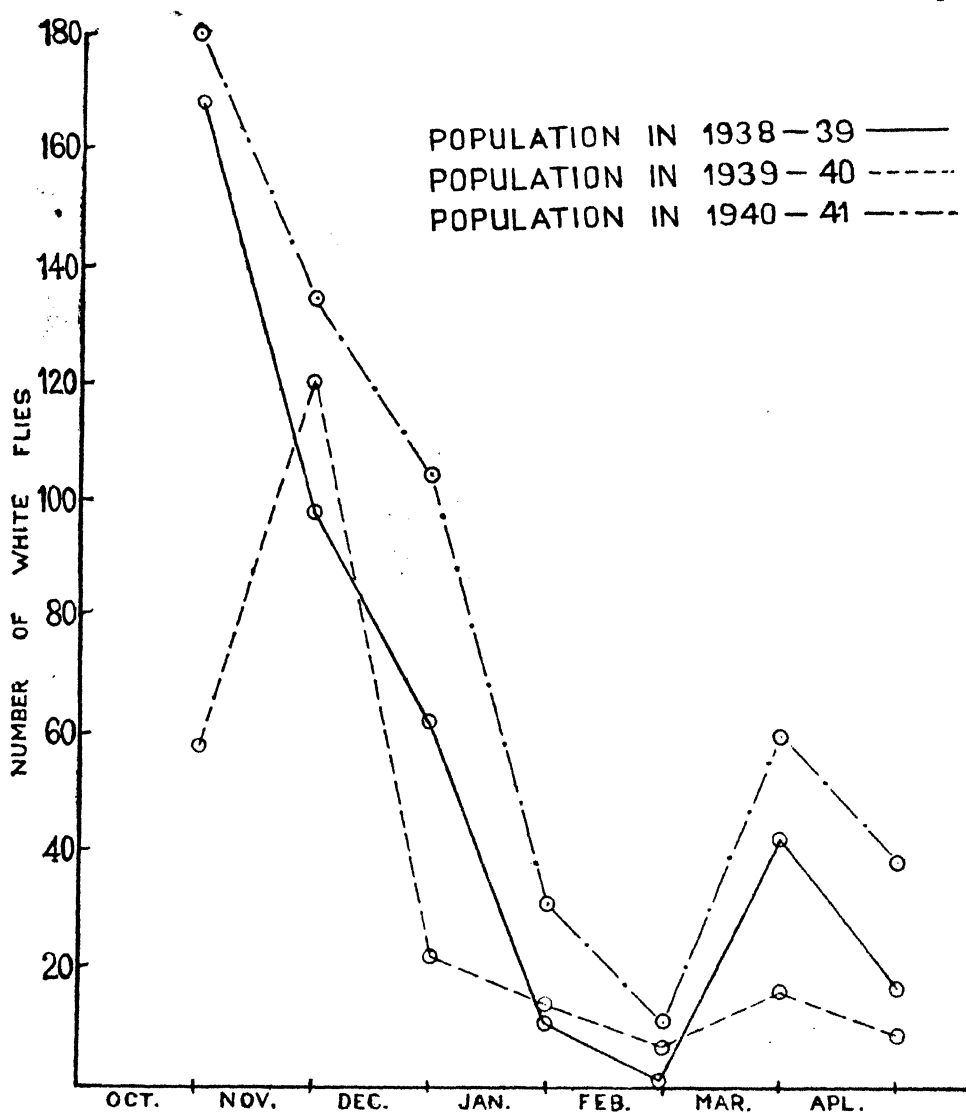


FIG. 1. The population of white-fly during 1938-41\*

When tobacco seedlings are transplanted in the field about the end of September, the white-flies, start migrating to them from duranta, sunn-hemp, *Ageratum*, *Launea*, and almost all the above named weeds. The migration of adults was observed in the fields from 7-30 to 10-30 A.M. and 2-30 to 5-30 P.M. in October-November. In the first two weeks of October, eggs were found on majority of the seedlings, while a large number of gravid females were still in the process of ovipositing. Hatching of the eggs took place in the field about the third week of October, and adults of the first brood appeared about the middle of November. The development of the

\* The number of white-flies given on the vertical axis are those found in a randomized area of three-quarters of an acre of tobacco plants.

white-fly is rather slow during December and January. Thus four to five generations only are completed on tobacco crop up to the end of March. As the vector has been observed to complete 12 generations in a year in the laboratory, it is obvious that the remaining generations are completed on its other hosts during March to the end of August. Thus the white-fly is able to thrive almost throughout the year on account of its having a wide host range. It overwinters in the form of pupae, of which some get actually killed by frost, fungus (*Alternaria* sp.), and parasites.

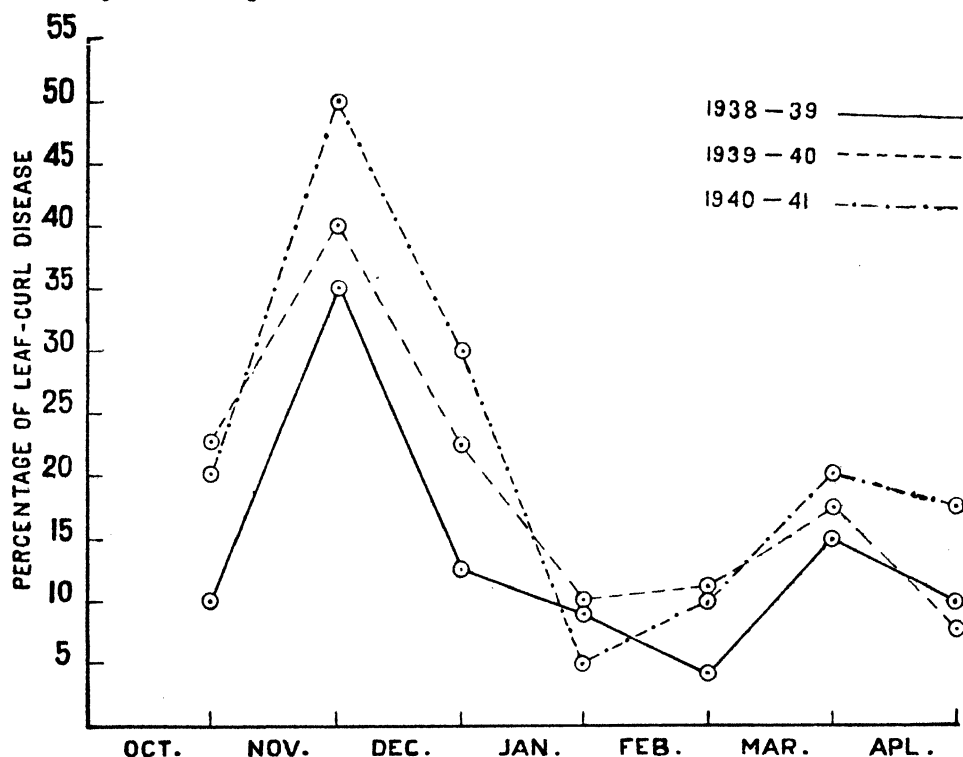


FIG. 2. The incidence of leaf-curl disease during 1938-41

Sticky boards were set up during August-December on the bunds of some fields, in which sunn-hemp, *urid*, soya-bean, *meth*, tobacco, etc. were growing, to note the extent and time of flights of the white-flies. The data collected showed that maximum number of captures were secured in September-October thus coinciding with the planting time of tobacco.

In 1938, a three-quarter acre tobacco plot (I P Hybrid 142) sown and transplanted respectively in September and October was selected for the purpose of estimating the changes in the population of the white-fly at different times of the year. The plot consisted of 44 rows with 110 plants in each row, and of these, to avoid border effect, 36 rows with 100 plants in each row were selected for taking the observations, the total number of plants under examination being 3,600. The plants were randomized, and about 72 plants distributed in 36 rows at the rate of two plants in each row were examined weekly. In addition to this, daily counts were also taken on 10 plants which had also been selected at random. The weekly population of

the white-fly was recorded by carefully examining all the leaves of the entire plants with a hand lens, and counting the number of different stages (nymphs, pupae and adults) of the vector present on each plant. Weekly counts of the leaf-curl plants were also recorded along with the population of the white-fly. Observations were taken on these lines throughout the tobacco season—beginning from the third week of October up to the middle of April. Similar observations were also taken during 1939-40 and 1940-41 in a field in which August-sown tobacco was transplanted in September or early in October (normal time). The data of incidence of the white-fly and leaf-curl collected during the three years are graphically shown in Figs. 1 and 2. From the data it is evident that the number of various stages as well as the adults decreased from the first week of November up to the second week of January, after which there was again an increase which continued up to the second week of April when the crop was harvested. The graphs in Figs. 3-5 show the meteorological data separately for the three years (1938-41) during which the incidence of the white-fly and leaf-curl disease was recorded.

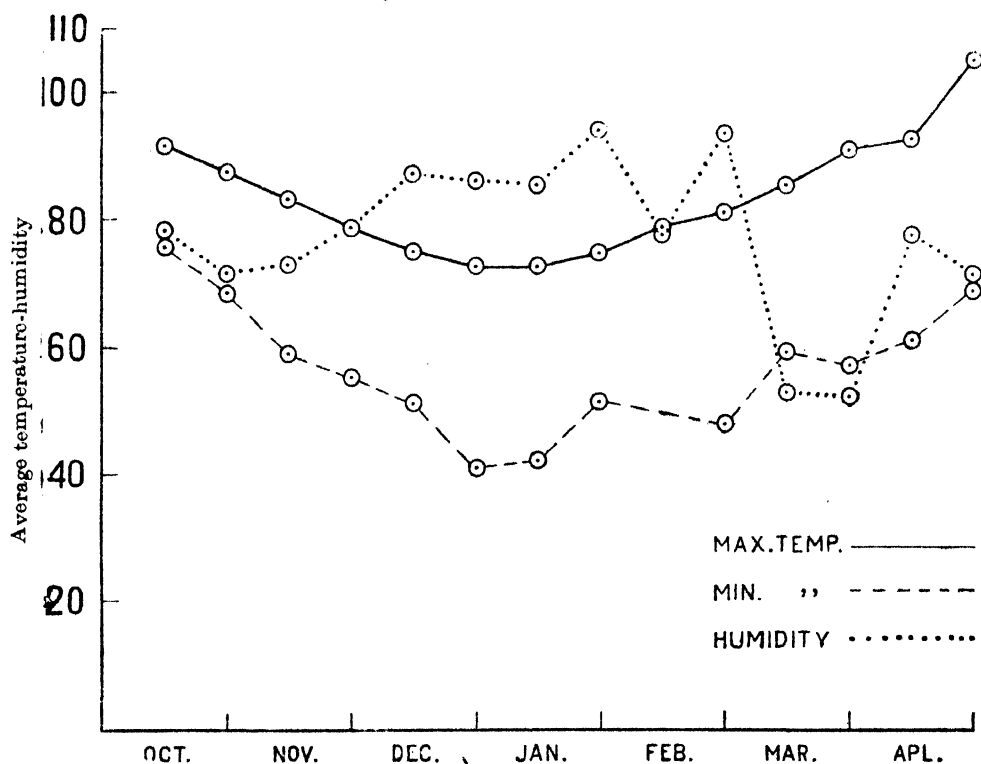


FIG. 3. Meteorological data\* during 1938-39

#### CORRELATION BETWEEN THE INCIDENCE OF WHITE-FLY AND TOBACCO LEAF-CURL

In order to ascertain whether any correlation existed between the incidence of the white-fly and the intensity of leaf-curl disease on tobacco crop,

\* The temperatures are given in degrees F.

seedlings were raised every month from July to December during 1939-40, and the seedlings from each nursery were successively transplanted in three-quarter acre plots. Weekly census of the white-fly (all stages) together with the incidence of diseased plants occurring in each plot every week were recorded. The data thus obtained are given in Table II.

In the case of July-August lot, the white-fly appeared in the fourth week of September and increased rapidly in numbers during October and November, but decreased from December onwards. The leaf-curl disease was first shown by eight plants in the last week of September, followed by a slight rise in October and November, but there was a considerable fall in December and January. Thus the intensity of leaf-curl corresponds with the rise and fall in the population of the white-fly. It may be pointed out that the incidence of leaf-curl in the fourth week of September shows that infection had already taken place in the nursery stage.

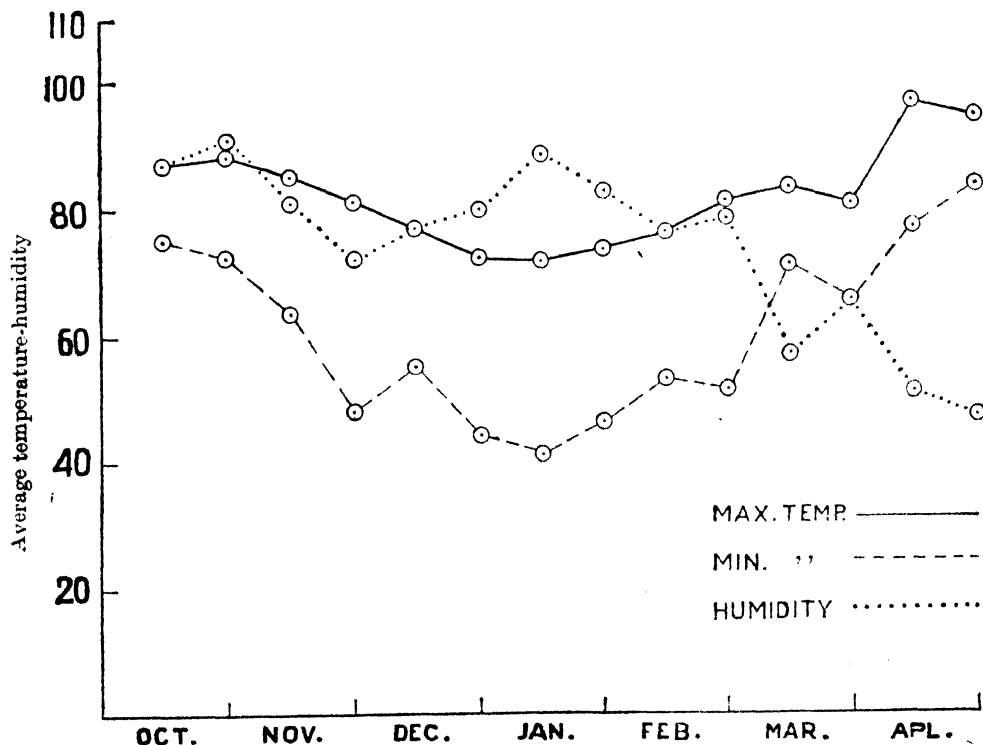


FIG. 4. Meteorological data\* during 1939-40

In the August-September lot, the white-fly appeared in large numbers in the second week of October. The incidence at the end of November was almost the same as in the July-August lot. It, however, decreased from December onwards. Diseased plants, which were first noticed in the third week of October decreased in number in November and December, and no fresh incidence of disease was observed in the rest of the season.

\* The temperatures are given in degrees F.

In the September-October lot, the white-fly appeared in the fourth week of October, but enormously increased in November, followed by a gradual decrease in the rest of the season. The corresponding incidence of leaf-cur which first appeared in November and December was practically negligible.

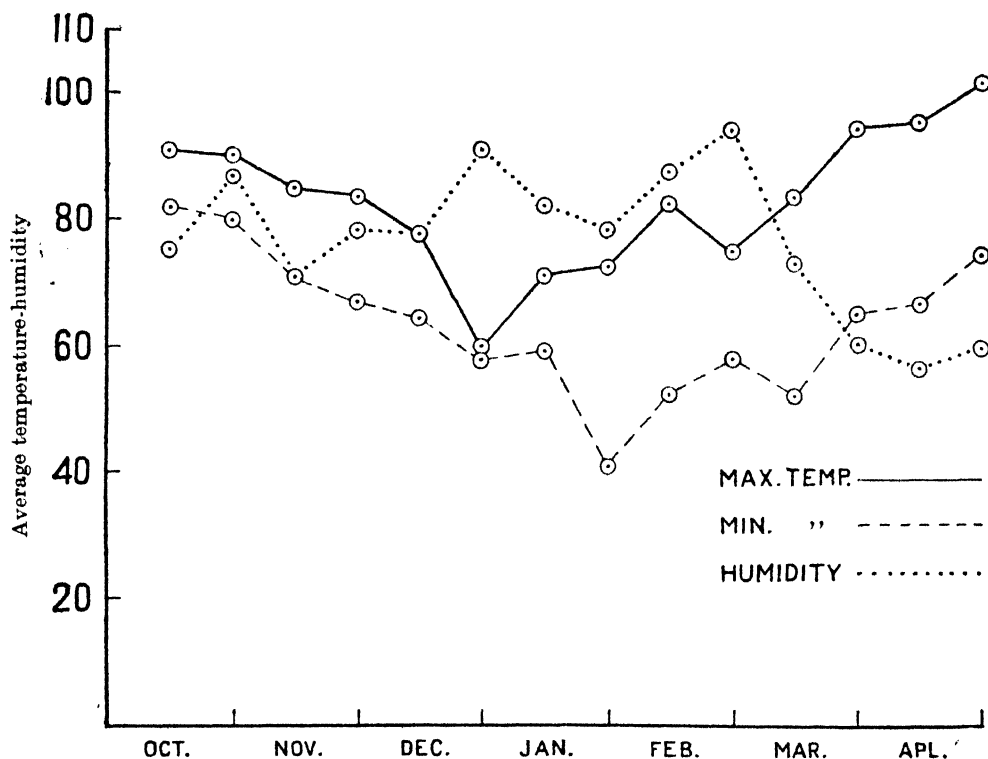


FIG. 5. Meteorological data\* during 1940-41

In the October-November lot, the white-fly began to appear in small numbers in the fourth week of December, and its incidence remained very low in the following months. Only two plants showed disease in the third week of December.

In the November-December lot, the low incidence of white-fly which was observed in the fourth week of January, remained almost steady up to March. The corresponding incidence of leaf-curl was also noticed to be very low.

In the December-January lot, white-fly began to appear in the first week of March and no increase was noticed in its numbers in April. The disease did not appear in the plot at all.

The foregoing observations show that the incidence of leaf-curl disease is closely dependent upon the population of the white-fly. Furthermore, it can be concluded that infection of seedlings in the nursery stage also takes place if they are kept exposed for a considerable time before being transplanted.

\* The temperatures are given in degrees F.



LEAF-CURL DISEASE OF TOBACCO IN NORTHERN INDIA, V

TABLE II  
Weekly incidence of white-fly and leaf-curl disease in the plot of periodical sowings of tobacco (I P H-142) during the crop year 1939-40

No.	Sowing Transplanting	Date of first appearance of the symptoms	September	October	November	December	January	February	March	April	Monthly total	GRAND TOTAL	Remarks
			1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4			
1	July-August— White-flies . . . Leaf-curl plants . . .	... 22-9-39	... 12 ... 8	19 45 21 35 2 1 1 2	86 23 7 6 6 1 3 2 2	9 1 5 2 ... 1 ... 1	... 6 ... 10 ... ... 1 1	5 ... ... ... ...	... 4 ... ...	... ... ... ...	4 ...	296 25	
2	August-September— White-flies . . . Leaf-curl plants . . .	... 15-10-39	... ... ... ...	... 11 15 32 ... 7 6 13	84 27 5 4 3 6 ...	3 1 6 12 9 1 ... ...	4 2 1 7 ... ...	2 5 ... ... ...	2 4 3 7 ... ...	5 4 ... ... ...	16 ...	246 23	Total number of plants under observation in each lot was 154.
3	September-October— White-flies . . . Leaf-curl plants . . .	... 19-11-39	... ... ... ...	... ... 9 ... ...	9 17 15 4 2 ... 1 ...	38 3 1 2 12 1 ... ... 1	1 2 ... 5 ... ...	7 2 ... 1 ... ...	... 6 6 ... ...	4 3 ... ... ...	14 ...	104 2	
4	October-November— White-flies . . . Leaf-curl plants . . .	... 26-12-39	... ... ... ...	... ... ... ...	... ... ... ...	... ... 1 ... ... 2	3 5 3 11 ... ...	6 ... ... ... ...	1 1 2 2 ... ...	... ... ... ...	6 ...	35 2	

Data for November and December sowings which were very few have not been shown in this table.

TABLE III

*Weekly incidence of white-fly and leaf-curt disease in the plot of periodical sowings of tobacco (I P H-142) during the crop year 1940-41*

No.	Sowing Transplanting*	Date of first appearance of disease symptoms	September				October				November				December				January				February				March				April				Monthly total	Grand total	Remarks																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
			1	2	3	4	Monthly total	1	2	3	4	Monthly total	1	2	3	4	Monthly total	1	2	3	4	Monthly total	1	2	3	4	Monthly total	1	2	3	4	Monthly total																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
1	July-August— White-flies { Exp. sds. Pro. sds. Leaf-curt plants { Exp. sds. Pro. sds.	...	...	3	2	5	6	4	7	...	17	1	3	1	1	6	1	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...</

Data for November and December sowings which were very few have not been shown in this table  
\* Exp. sds.—Exposed seedlings; Pro. sds.—Protected seedlings



The above observations were continued during the year 1940-41, when, unlike the previous year, nursery seedlings were raised under two sets of conditions, viz. one set was exposed to nature, while the other was protected under an insect-proof cover up to the time of transplanting. The data thus collected are given in Table III.

From a close examination of the data it is evident that as in the case of the previous two years, the incidence of the disease was dependent on the population of the white-fly, and further seedlings under insect-proof covers in the nursery showed lower incidence of the white-fly and correspondingly lower incidence of the disease than those which were exposed.

#### NATURAL ENEMIES

An unidentified Chalcid parasite of the pupa of *Bemisia tabaci* on cotton has been recorded by Husain and Trehan [1933]. At Pusa three new Chalcid parasites, viz. *Prospaltella smithi* Silv., *Pteropteryx bemisiae* Mani (to be described elsewhere) and *Eretmocerus masii* Silv., were noticed parasitizing the pupa of this white-fly while infesting several food-plants, viz. tobacco, sunn-hemp, *Ageratum*, soya-bean, duranta, cowpea, gingelly, chillies, zinnia, cotton, etc. The parasites were noticed during the months of November-January, and of the three species, *P. bemisiae* appeared to be most common.

Parasitized pupae could be easily distinguished from the normal ones by the dark colour of their entire body except the vasiform end which was reddish-brown. The pupal cases of the parasitized white-fly left after the emergence of the parasites and white-flies can also be easily distinguished. They have a tiny circular hole in the region of the thorax on the dorsum in addition to dark or reddish-brown colouration which very often persists even after the emergence of the parasites.

In order to estimate the degree of parasitization of the host on these various plants, the leaves from each plant were collected at random and the parasitized and non-parasitized pupae were counted on each leaf. The parasitism was found to vary from 1.5 to 5.1 per cent. On cotton, where intense breeding of the white-fly in the rearing cages was noticed, parasitism was found to be as high as 62.73.5 per cent.

#### SUMMARY

1. The biology of the white-fly, *Bemisia tabaci*, the vector of the leaf-curl virus disease of tobacco, was studied in tobacco fields at Pusa for five years.

2. The white-fly has a large number of alternate food-plants, some of which also suffer from virus diseases and have been proved to be the alternate hosts of the tobacco leaf-curl virus. A complete list of the food-plants with the time of the year when the white-fly is found on them and its intensity is given in Appendix I.

3. The population of the white-fly on tobacco crop studied at different times of the year showed that it is highest in autumn (up to the middle of November), goes down in winter and rises again in March.

4. The incidence of the disease in tobacco is dependent on the population of the white-fly.

5. A brief account of the natural enemies of the white-fly is given.

## ACKNOWLEDGEMENTS

Our sincere thanks are due to the Imperial Economic Botanist for kindly allotting a tobacco plot for our observations, besides many other facilities provided for our work at the Botanical Sub-station, Pusa. The Imperial Mycologist has kindly identified the entomogenous fungus found infesting the pupae of the white-fly.

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## APPENDIX I

*Food-plants of the white-fly, Bemisia tabaci Gen., in the environs of Pusa (Bihar) and remarks on the incidence of leaf-curl disease in them*

Food-plant	Time of occurrence of the white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
<b>SOLANACEÆ</b>		
<i> Capsicum annuum</i> *	Oct.-Dec.; Feb.-March ; severe in Feb.-March.	Oct.-Dec.; 15-25 per cent
<i>Datura stramonium</i> *	Feb.-March ; moderate	Sept.-Oct.; 75 per cent
<i>Hyoscyamus niger</i>	Oct.-Feb.; severe	....
<i>Lycopersicum esculentum</i> **	Feb.-March ; moderate	Aug.-March ; 6-15 per cent
<i>Lycopersicum pimpinellifolium</i> *	Oct.-Dec.; moderate	Nov.-Dec.; 1 per cent
<i>Nicandra physaloides</i> *	Nov.-Dec.; moderate	Oct.-Dec.; 2 per cent
<i>Nicotiana glauca</i>	Oct.-Nov.; moderate	....
<i>Nicotiana glutinosa</i>	Ditto	....
<i>Nicotiana plumbaginifolia</i>	....	....
<i>Nicotiana rustica</i> *	Oct.-Nov.; moderate	Severe in Oct.-Nov.; 25-30 per cent
<i>Nicotiana tabacum</i> **	Aug.-Dec.; severe in Nov.	Aug.-Dec.; 30-40 per cent
<i>Petunia phoenicea</i>	Oct.-Nov.; moderate	....
<i>Physalis angulata</i> *	Nov.-Jan.; moderate	Dec.; trace
<i>Physalis peruviana</i> *	Oct.-Dec.; severe	Oct.-Dec.; 40 per cent
<i>Solanum melongena</i> *	Oct.; moderate	Oct.-Nov.; 1 per cent
<i>Solanum nigrum</i> **	Oct.-Nov.; Feb.-March ; moderate.	July ; March ; 5 per cent
<i>Solanum tuberosum</i> *	Oct.-Dec.; Feb. moderate	Nov.-Jan.; 2-5 per cent
<i>Solanum verbascifolium</i>	Aug.-Oct.; moderate	....
<b>LEGUMINOSÆ</b>		
<i>Arachis hypogaea</i> *	June-Aug.; moderate	July-Aug.; 5-12 per cent
<i>Cajanus cajan</i> *	Dec.-Feb.; Aug.-Oct.; very low	Dec. ; trace
<i>Cicer arietinum</i> *	Dec.-Jan.; very low	Dec.-Jan.; 5-10 per cent
<i>Clitoria ternatea</i>	March-April ; very low	....

\* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

\*\* The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX I—*contd.*

Food-plant	Time of occurrence of the white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
LEGUMINOSEÆ— <i>contd.</i>		
<i>Crotalaria juncea</i> **	June-Oct.; severe	Aug.-Nov.; 10-15 per cent
<i>Dolichos lablab</i>	Oct.-Dec.; very low	....
<i>Ervum lens</i>	Dec.-Jan.; very low	....
<i>Glycine hispida</i> *	Oct.-Nov.; moderate	Oct.-Nov.; trace
<i>Medicago sativa</i>	March-April; very low	....
<i>Melilotus parviflora</i>	Sept.-Oct.; very low	....
<i>Phaseolus calcaratus</i> *	Oct.-Nov.; March-April; moderate	Oct.-Nov.; trace
<i>Phaseolus mungo</i> *	March-April; severe	March-April; 70-80 per cent
<i>Phaseolus radiatus</i> *	July-Oct.; moderate	Aug.-Sept.; 30-40 per cent
<i>Phaseolus vulgaris</i>	Dec.-Jan.; very low	....
<i>Pisum arvense</i>	Ditto	....
<i>Pisum sativum</i>	Ditto	....
<i>Trifolium alexandrinum</i>	March-April; low	....
<i>Vigna catjang</i>	Oct.-Dec.; very low	....
COMPOSITÆ		
<i>Ageratum conyzoides</i> **	July-Nov.; moderate	July-Dec.; 20-25 per cent
<i>Calendula officinalis</i> *	Dec.-Jan.; very low	Dec.-Jan.; 1-2 per cent
<i>Carthamus tinctorius</i> *	Dec.-Mar.; fairly severe	Dec.-Feb.; 10 per cent
<i>Cosmos bipinnatus</i> *	Dec.-Jan.; very low	Dec.-Jan.; 1-2 per cent
<i>Inula (Vicoa) vestita</i> *	Aug.-Sept.; Nov.-Dec.; very low	Dec.-Mar.; 30-40 per cent
<i>Launea asplenifolia</i> **	Jan.-Mar.; June-Sept.; severe in Feb.-March	July-Mar.; 50 per cent
<i>Vernonia anthelmentica</i> *	Sept.-Dec.; moderate	Sept.-Nov.; 5-10 per cent
<i>Vernonia cinerea</i> **	July-Oct.; very low	July-Feb.; 5-10 per cent
<i>Xanthium strumarium</i>	Jan.-Mar.; moderate	....
<i>Zinnia elegans</i> **	Aug.-Oct.; moderate	Aug.-Jan.; 15-20 per cent

\* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

\*\* The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX I—*contd.*

Food-plant	Time of occurrence of the white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
<b>MALVACEÆ</b>		
<i>Althaea rosea</i> * . . .	Aug.-Dec.; Jan.-April ; moderate	Aug.-Dec.; 5-15 per cent
<i>Gossypium herbaceum</i> . . .	Ditto . . .	....
<i>Hibiscus cannabinus</i> * . . .	July-Nov.; moderate . . .	Aug.-Dec.; 10-15 per cent
<i>Hibiscus esculentus</i> * . . .	Aug.-Oct.; moderate . . .	Aug.-Dec.; 5-10 per cent
<i>Hibiscus rosa-sinensis</i> * . . .	Aug.-Oct.; Feb.-March ; moderate	All round the year ; 40-50 per cent
<i>Sida cordifolia</i> . . .	Aug.-Oct.; low . . .	....
<i>Sida rhombifolia</i> ** . . .	Aug.-Oct.; moderate . . .	July-Feb.; 5-15 per cent
<b>LINACEÆ</b>		
<i>Linum usitatissimum</i> . . .	Sept.-Nov.; low . . .	....
<b>CRUCIFERÆ</b>		
<i>Brassica campestris</i> . . .	Nov.-Jan.; very low . . .	....
<i>Brassica napus</i> * . . .	Ditto . . .	Nov.-Feb.; 10-15 per cent
<i>Brassica oleracea</i> * . . .	Ditto . . .	Ditto
<i>Brassica oleracea</i> var. <i>botrytis</i> * . . .	Ditto . . .	Ditto
<i>Brassica juncea</i> . . .	Ditto . . .	....
<i>Brassica rapa</i> * . . .	Ditto . . .	Nov.-Feb.; 2-5 per cent
<i>Raphanus sativus</i> * . . .	Ditto . . .	Nov.-Feb.; 5-10 per cent
<b>CUCURBITACEÆ</b>		
<i>Cucumis melo</i> . . .	April-May ; low . . .	....
<i>Cucumis sativus</i> * . . .	Aug.-Oct.; low . . .	Sept.-Nov.; 1 per cent
<i>Lagenaria vulgaris</i> * . . .	July-Sept.; low . . .	Oct.-Nov.; 1 per cent
<i>Luffa acutangula</i> * . . .	Ditto . . .	Sept.-Oct.; trace
<i>Luffa aegyptiaca</i> * . . .	Ditto . . .	Ditto
<i>Trichosanthes anguina</i> * . . .	Ditto . . .	Ditto
<i>Trichosanthes dioica</i> . . .	Sept.-Nov.; March-April; moderate	....

\* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

\*\* The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX I—*contd.*

Food-plant	Time of occurrence of the white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
<b>UMBELLIFERÆ</b>		
<i>Coriandrum sativum</i> . . .	Jan.-Feb.; low . . .	....
<b>LABIATÆ</b>		
<i>Anisomeles ovata</i> * . . .	Aug.-Oct.; moderate . . . Feb. ;	Sept.-Nov.; trace
<i>Nepeta ruderalis</i> . . .	Aug.-Nov.; moderate . . . Feb. ;	....
<i>Ocimum basilicum</i> . . .	Aug.-Oct.; low . . .	....
<i>Ocimum sanctum</i> . . .	Ditto . . .	....
<b>EUPHORBIACEÆ</b>		
<i>Euphorbia hirta</i> ** . . .	July-Sept.; March-April; low . . .	July-Dec.; 5-10 per cent
<i>Euphorbia heterophylla</i> . . .	Aug.-Oct.; March-April; moderate . . .	....
<i>Euphorbia hypericifolia</i> . . .	Ditto . . .	....
<i>Euphorbia prostrata</i> . . .	Ditto . . .	....
<b>CONVOLVULACEÆ</b>		
<i>Convolvulus arvensis</i> . . .	Oct.-Dec.; very low . . .	....
<i>Coccinia indica</i> . . .	Ditto . . .	....
<i>Ipomœa batatas</i> * . . .	Oct.-Dec.; moderate . . .	Nov., trace
<i>Ipomœa reptans</i> . . .	Ditto . . .	....
<b>AMARANTACEÆ</b>		
<i>Achyranthes aspera</i> * . . .	Aug.-Sept.; moderate . . . Nov.-Dec.;	Aug.-Dec.; 5-12 per cent
<i>Amarantus gangeticus</i> . . .	Sept.-Nov.; very low . . .	....
<i>Amarantus spinosus</i> . . .	Ditto . . .	....
<i>Amarantus viridis</i> . . .	Ditto . . .	....
<i>Celosia cristata</i> . . .	Ditto . . .	....

\* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

\*\* The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX I—*concl'd.*

Food-plant	Time of occurrence of white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
<b>CAPPARIDÆ</b>		
<i>Oleome chelidoniæ</i> . . .	Sept.-Oct.; very low .	....
<i>Oleome viscosa</i> . . .	Ditto . .	....
<b>ACANTHACEÆ</b>		
<i>Ruellia prostrata</i> . . .	July-Sept.; low . .	....
<i>Sesamum indicum</i> * . . .	Sept.-Nov.; moderate .	Sept.-Oct.; 8-15 per cent
<b>VERBENACEÆ</b>		
<i>Clerodendron infortunatum</i> * .	Aug.-Oct.; very low .	Sept.-Dec.; 10-25 per cent
<i>Duranta plumieri</i> * . . .	Oct.-Jan.; March-April ; moderate	Oct.-Jan.; 5-20 per cent
<b>TILIACEÆ</b>		
<i>Corchorus capsularis</i> . . .	Oct.-Dec.; moderate .	....
<i>Corchorus acutangulus</i> . . .	Ditto . .	....
<b>URTICACEÆ</b>		
<i>Cannabis sativa</i> * . . .	Feb.-April ; moderate .	Feb.-March.; 1-2 per cent
<b>CHENOPODIACEÆ</b>		
<i>Chenopodium album</i> . . .	Oct.-Dec.; very low .	....
<b>SCROPHULARINEÆ</b>		
<i>Scoparia dulcis</i> ** . . .	July-Oct.; moderate .	July-Dec.; 10-15 per cent
<b>ROSACEÆ</b>		
<i>Rosa centifolia</i> . . .	Feb.-April ; very low .	....
<b>GERANIACEÆ</b>		
<i>Oxalis corniculata</i> . . .	Sept.-Nov.; very low .	....
<b>GRAMINACEÆ</b>		
<i>Cymbopogon burmanni</i> . . .	Sept.-Dec.; fairly high .	....
<b>COMMELNACEÆ</b>		
<i>Commelina benghalensis</i> . .	Sept.-Dec.; fairly high .	....

\* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

\*\* The virus on these plants is the same which causes leaf-curl in tobacco.

## APPENDIX II

*Meteorological data for the periods during which oviposition records were taken*

Year	Average temperatures during the period (°F.)		Relative humidity (per cent)	Remarks
	Maximum	Minimum		
1936—				
30 August—4 September . . . . .	88·8	80·2	91·0	
23—29 September . . . . .	89·8	77·2	87·0	
17—27 October . . . . .	84·0	70·4	82·0	
21 November—12 December . . . . .	78·5	49·0	82·0	
1937—				
7—21 January . . . . .	74·7	42·5	72·0	
17—26 February . . . . .	68·3	55·5	94·0	
25 March—4 April . . . . .	93·5	66·5	51·0	
20—27 April . . . . .	101·5	70·5	49·0	
17—23 May . . . . .	103·7	74·5	61·0	
14—22 June . . . . .	..	..	..	Temperature records not taken
9—15 July . . . . .	..	..	..	
6—11 August . . . . .	91·0	79·0	83·0	
1—9 September . . . . .	92·0	80·0	80·0	
2—10 October . . . . .	83·0	70·0	89·0	
2—13 November . . . . .	82·8	61·5	78·0	
6—20 December . . . . .	70·5	43·0	83·0	
1938—				
16 January—1 February . . . . .	74·0	47·5	84·0	
21 February—3 March . . . . .	74·9	46·0	74·0	
28 March—4 April . . . . .	96·0	60·5	40·0	
24 April—1 May . . . . .	97·0	73·5	48·0	

APPENDIX II—*concl.*

Year	Average temperatures during the period (°F.)		Relative humidity (per cent)	Remarks
	Maximum	Minimum		
1938— <i>contd.</i>				
5—12 May . . . . .	93·0	70·5	84·0	
17—23 May . . . . .	95·0	78·5	72·5	
25 May—4 June . . . . .	90·0	78·0	87·0	
12—19 June . . . . .	93·5	77·0	93·0	
19—25 June . . . . .	93·0	79·3	78·0	
6—12 July . . . . .	91·5	80·5	85·0	
12—19 July . . . . .	88·5	78·2	91·0	
6—12 August . . . . .	87·5	78·2	93·0	
24—30 August . . . . .	91·5	81·0	80·0	
10—21 September . . . . .	93·5	79·2	89·0	
3—14 October . . . . .	92·4	76·7	79·0	
4—15 November . . . . .	84·0	59·7	76·0	
10—24 December . . . . .	74·5	47·2	94·0	
1939—				
25 January—9 February . . . . .	75·5	53·0	94·0	
20—28 March . . . . .	93·0	59·5	49·0	
16—24 April . . . . .	95·0	62·5	46·0	

# BIOLOGICAL CONTROL OF THE COTTON STEM WEEVIL, *PEMPHERULUS AFFINIS* FST., IN SOUTH INDIA

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(With Plate IV and ten text-figures)

*PEMPHERULUS AFFINIS* Fst. commonly known as the cotton stem weevil and one of the major pests of both exotic and indigenous varieties of cotton in south India, is widely distributed in the cotton-growing districts of Madras. With a view to furthering the possibilities of its control, the Indian Central Cotton Committee sanctioned a small scheme to study its distribution in India, both on cotton and its alternative hosts, in conjunction with a search for parasites and predators. The scheme was put into operation in October 1935 and continued for a period of three years. The present paper records the results obtained in this preliminary investigation.

## METHODS

At the outset the need for exact information on the incidence, habits and reactions of the stem weevil was felt. Continuous and quantitative field studies were made to trace the annual course of weevil-breeding, with particular reference to the time and character of its incidence in cotton. Brief surveys of the important cotton-growing tracts and quantitative collections and examinations of alternate host plants were also undertaken. In the course of these studies over 55,000 cotton plants, 23,000 alternate host plants and 14,000 specimens of parasites have been handled.

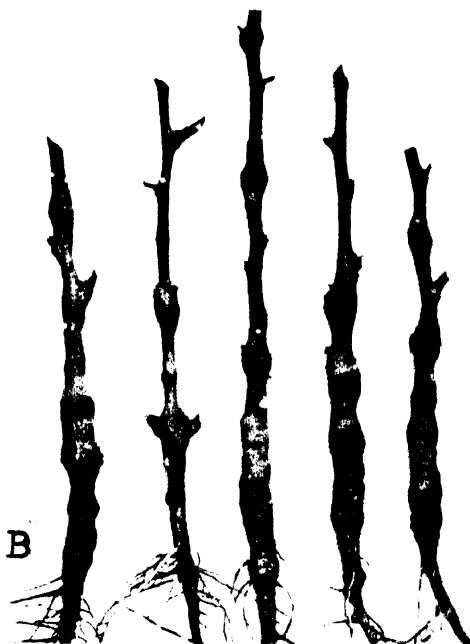
The mass of data accumulated during the period may be conveniently dealt with under three main heads: (1) studies on the weevil, (2) studies on parasites and predators, and (3) a concluding part summarizing the present position of our knowledge of the problem.

## PREVIOUS KNOWLEDGE

The information so far available may be briefly summarized as follows. Ramakrishna Ayyar [1918] and Ballard [1922] published general outlines of the life-history and the different stages of the insect. It was then known to occur only in Coimbatore, Salem, Trichinopoly, Madura, Ramnad and Malabar districts. The life-cycle is recorded to range from 77 to 99 days: 6-8 days as egg, 35-57 days as larva and 9-10 days as pupa. The life-span of the adult is recorded to be about 36 days. The average egg-laying capacity is about 15.5 eggs per female with a maximum of 30. The emergence period extends to about a month. Among its alternate host plants are included a dozen species, such as *Althaea rosea*, *Hibiscus esculentus*, *H. cannabinus*, *Corchorus olitorius*,



A



B



C



D

- A. A typical *Pempherulus*-infested Cambodia cotton plant.
- B. Cotton stems depicting variations in number, nature, position, shape and size of gall formation.
- C. Cotton stems with bark peeled off showing early course of attack and tunnelling round the stems.
- D. Longitudinal section through infested cotton stems revealing the damage caused inside the stems by the grubs.



*Sida spinosa*, *Triumfetta* spp., *Ficus religiosa*, *Hibiscus rosa-sinensis*, *Calotropis gigantea*, *Melia azadirachta*, *Abutilon indicum* and *Dombeya*. No natural enemies, either parasites or predators, were known. Indigenous varieties of cotton were supposed to be less susceptible to weevil attack.

## I. STUDIES ON THE WEEVIL

### DISTRIBUTION

#### Madras Province

As these studies were based on brief reconnaissance tours in certain selected cotton-growing tracts in the province the data cannot be regarded as either exhaustive or conclusive. Broadly speaking, the distribution of the pest is restricted to the southern parts of the Madras province, where it has developed into a major pest of cotton. In the northern districts the weevil may be considered to be almost absent as a pest of cotton, its place being, in a way, taken up by the Buprestid borer, *Sphenoptera gossypii*, though far less destructive. To be more precise, *Pempherulus* is now definitely known to occur either in cotton or allied food plants in the whole of Coimbatore district, almost the whole of Ramnad district, the southern portion of Tinnevely, the whole of Malabar and the southern border of south Canara and portions of the adjoining native states of Cochin and Travancore. In Malabar and south Canara the insect has been noted only on allied food plants but not cotton. Stray specimens of the weevil have also been received from a few of the northern districts like Vizagapatam. It is practically absent in the Ceded Districts.

#### Other parts of India

A brief visit to Bihar, United Provinces, Gujerat and Hyderabad (Deccan) forms the basis for these observations. The weevil is not known as a serious pest of cotton in any of these provinces in India. It has, however, been noted to breed in alternate host plants like *Hibiscus esculentus* at Pusa and Sasamusa at Surat and Dehra Dun. From Dehra Dun it has also been found on other allied plants such as *Urena lobata*, *Althaea rosea*, *Sida rhombifolia* and *S. acuta*. The weevil has not so far been observed in cotton or alternate food plants from Hyderabad.

#### Distribution outside India

The species is reported to infest cotton in Burma and Philippines. It has been ascertained that the weevil is absent in Indo-China, Federated Malay States, Australia, New Zealand, Brazil, British West Indies, South Africa, Rhodesia, Uganda, Sudan, Egypt, U. S. S. R. and Ceylon.

#### The genus *Pempherulus* and its distribution\*

The genus *Pempherulus* comprises only five known species whose distribution is confined to the Indo-Malayan zone as may be seen from the data furnished.

*P. affinis* Fst.—India, Burma and Philippines

*P. habena* Pascoe.—Singapore, Malacca and Philippines

\* The writer is indebted to the Imperial Institute of Entomology, London, and U. S. Bureau of Entomology, Washington, for this information.

*P. pleurostigma* Fst.—South India

*P. picta* Heller.—Tenasserim (Lower Burma)

*P. trilineata* Pascoe.—Batchian (Dutch East Indies)

Three of these species are restricted to Indo-Burman region. The genus may be regarded as Indian or Indo-Burman in origin.

#### SEASONAL HISTORY

##### *Trend of incidence in seasonal crops*

A systematic collection of cotton plants and detailed examination of the same week after week and month after month were arranged. The data were recorded in a definite and uniform plan so as to afford an idea of the progress of incidence, seasonal history, number of generations, density of populations, course of parasitism and other controlling factors. The material collected cannot be considered thoroughly representative, owing to limitations of field area and staff, and also to the inevitable factor of the erratic distribution of the pest. Notwithstanding these handicaps, the quantitative data secured during the period indicated the main trends.

TABLE I  
*Percentage infestation in seasonal crop*

Month	1935-36		1936-37		1937-38	
	No. of plants examined	Percent-age of infestation	No. of plants examined	Percent-age of infestation	No. of plants examined	Percent-age of infestation
September .	1,200	0·0	..	..	..	..
October .	3,158	3·2	224	1·3	1,470	12·85
November .	803	23·2	832	4·7	2,302	14·80
December .	1,381	28·4	539	68·0	727	35·40
January .	1,228	55·0	684	83·0	2,292	54·00
February .	1,577	77·2	2,278	89·9	1,464	57·00
March .	869	91·7	345	90·1	668	59·00
April .	498	88·0	321	93·5	..	..
May .	455	99·0	109	98·2	..	..
June .	154	100·0	79	98·7	..	..
July .	260	100·0	..	..	..	..
August .	210	100·0	..	..	..	..

An analysis of the data recorded (Table I) for the three years reveals a general uniformity in the trend of infestation from October to March. It reveals that weevil incidence grows in intensity as the season advances. The seasonal history is roughly characterized by the occurrence of three generations though considerably overlapping from October to March. The first of these generations roughly covers the period from October to December. The second is not very clearly defined but occupies a period up to middle of February.

By this period the overlapping of broods is so heavy that generations are almost indistinguishable. A third brood may commence in February and extend far into April. Beyond this period, owing to heavy overlapping, broods are absolutely indistinguishable. Despite this overlapping, two more supplementary broods, though feeble, could be indistinctly traced, if the crop is retained till the end of August.

Though these general remarks are applicable to the three seasons under review, there have been considerable variations in the nature and extent of infestation and progress during different seasons. In the early stages of the crop, say within three to four weeks, there is a total absence of infestation as may be seen from 0 per cent in September. The fresh initial wave of incidence is visible in October and the progress of this brood is distinct. Oviposition probably commences during the month and probably to a slight extent by the latter part of the previous month and stray, newly hatched grubs form the only noticeable stages during the period. The percentage of infestation ranged from 1.3 to 12.85. Small incipient galls are apparent even at this stage.

Early part of November reveals the presence of medium-sized grubs, a proportion of which reaches maturity towards the close of the month. The percentage shows an increase and ranges from 4.7 to 23.2. All stages including prepupae, pupae and adults along with emergence apertures and large well-formed galls are available in December when the percentages vary from 28.4 to 68. The first generation is now almost nearing completion and the partial wave of emergence may be noticed. By January the second generation may be said to have commenced with the attack ranging from 54 to 83 per cent. In spite of three successive broods in the season, the live population does not show a steady increase throughout. Towards the middle of February the live population reaches its peak, varying from 45.4 to 98.5 stages per 100 plants (1937 and 1938). Thereafter, the population shows a gradual decline, ranging from 15.7 to 28.7 (Table II).

TABLE II

*Trend of live population*  
(Live population per 100 plants)

Month	1936-37	1937-38
October . . . . .	1.3	13.1
November . . . . .	4.1	10.7
December . . . . .	72.9	31.8
January . . . . .	68.2	42.7
February . . . . .	98.5	45.4
March . . . . .	28.7	15.7

*Possible factors.*—The actual causes of the fall in population are, however, not definitely known. On the other hand it is obvious that only successful waves of adult emergence at every generation can maintain or augment population density.

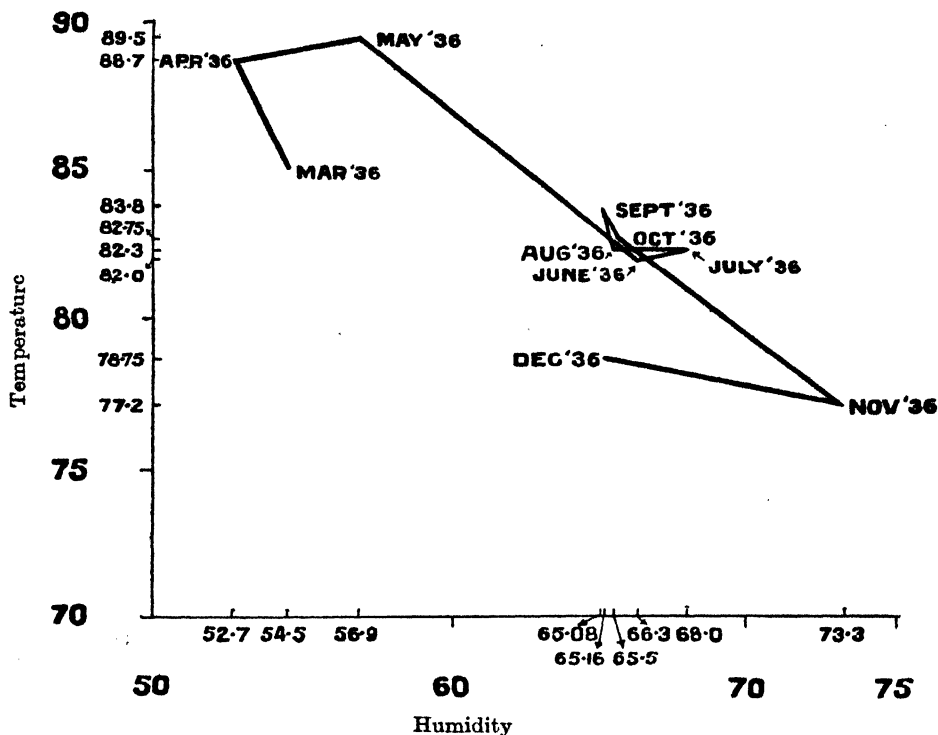


FIG. 1. Temperature-humidity curve, 1936

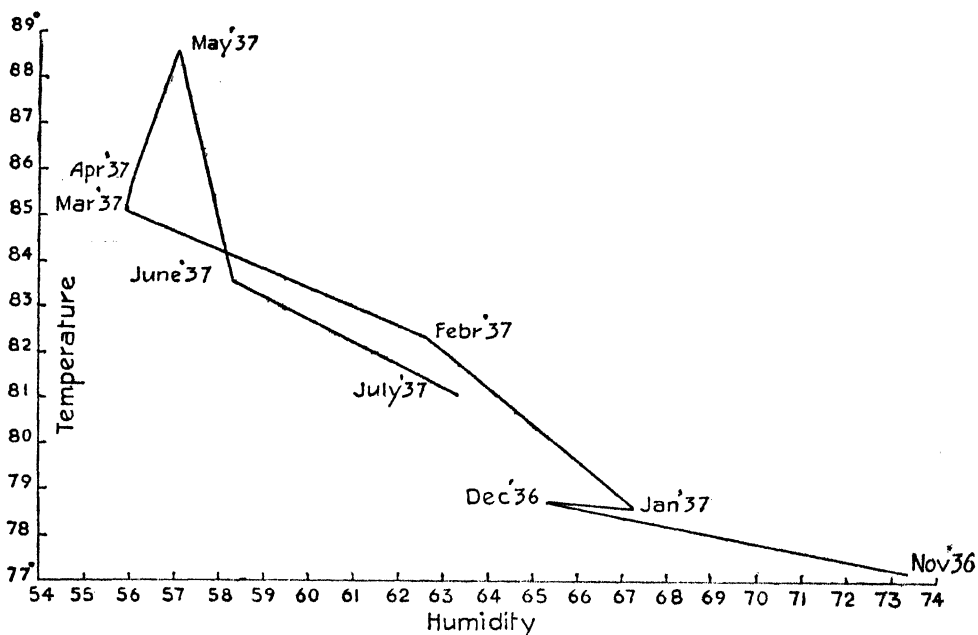


FIG. 2. Temperature-humidity curve, November 1936 to July 1937

But the prevailing ecological conditions at this stage seem to operate against this. Among these, the increased resistance inherent in the plants by pronounced proliferation and gumming which showed a marked increase during the reproductive phase of the plant, may be counted as one. Another probable factor in operation may be the unsuitability of the crop due to changes in plant constitution. This has in a way been corroborated by chemical analysis. Finally, the prevailing dry spell due to higher temperatures and lower humidities (Figs. 1, 2 and 3) and the slight increase in the parasitic element (Tables VIII and IX) may also have had their share in contributing to this decline.

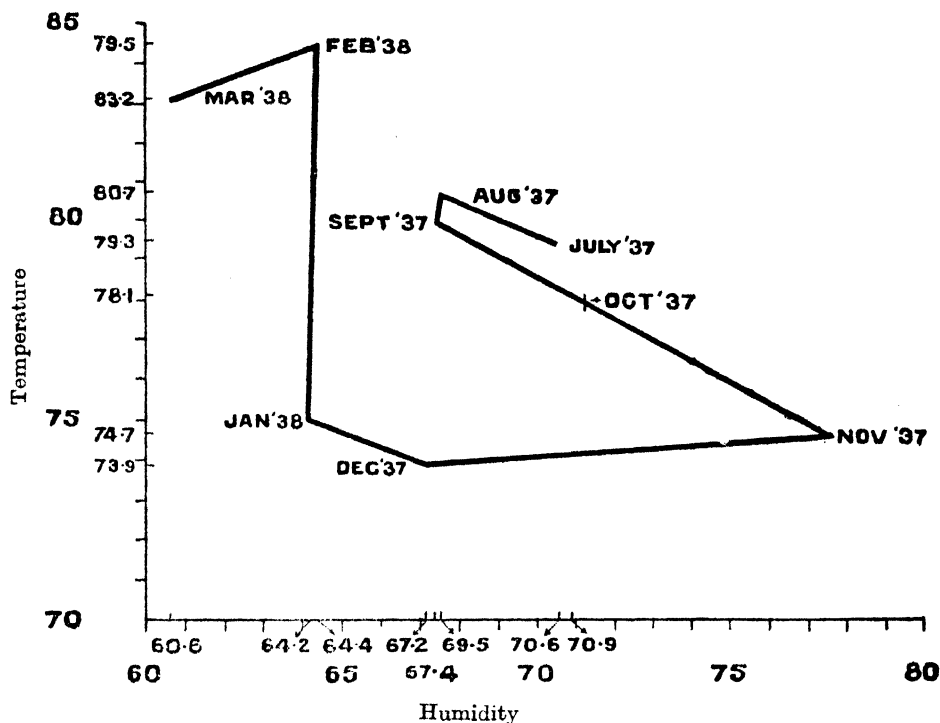


FIG. 3. Temperature-humidity curve, 1937-38 (average mean)

#### *Trend of incidence in off-seasonal crops*

The decrease in the density of population in the seasonal crop by about February raised the question of the influence of the season marked by high temperatures and low humidities as compared with the age of the crop. With a view to shedding some light on this problem, an off-seasonal crop was raised for the first time in February 1937 and again in 1938. From Table III it may be noted that there was a phenomenally heavy incidence and parasitism in 1937, which served to suggest the importance of the age of the crop as against physical factors. The data for 1938 have not been significant for the purpose. It may, therefore, be seen that no satisfactory answer can be given unless the experiment is repeated for a series of years and correlated with meteorological data.

TABLE III  
*Percentage infestation in off-seasonal crops*

Month	1937		1938	
	No. of plants examined	Percentage of infestation	No. of plants examined	Percentage of infestation
March . . . . .	640	5·6	..	..
April . . . . .	300	36·7	676	10·9
May . . . . .	253	79·0	570	22·1
June . . . . .	324	100·0	1,268	29·3
July . . . . .	632	100·0	2,154	47·0
August . . . . .	1,355	100·0	1,588	87·8
September . . . . .	no crop		767	93·7

*Status of the weevil as pest of cotton*

A systematic collection and examination of all dead plants from the fields afford striking proof of the status and destructiveness of the weevil. From the data furnished (Table IV) nearly 97 per cent of the total mortality in the crop is caused by *Pempherulus*.

TABLE IV  
*Mortality in plants*

Month	Number of plants	Percentage
November 1935 . . . . .	1,087	95·0
December 1935 . . . . .	590	94·6
January 1936 . . . . .	380	98·5
February 1936 . . . . .	657	100·0
March 1936 . . . . .	325	98·8
April 1936 . . . . .	205	100·0
May 1936 . . . . .	179	100·0
June 1936 . . . . .	60	100·0
July 1936 . . . . .	31	100·0
November 1936 . . . . .	700	98·0
December 1936 . . . . .	300	100·0
January 1937 . . . . .	200	100·0

*Influence of temperature and humidity on the weevil*

The irregular distribution of the insect in different years and the remarkable variation in incidence in different localities necessitated an extensive study of its reactions under different combinations of temperature and humidity. Apparently its heavy incidence in certain localities indicated that there are definite optimum localities having special environmental conditions, particularly temperature and humidity. Therefore, a study of its physical ecology was undertaken. The temperature was controlled by using different capsules in an electric incubator. Humidities were adjusted by using sulphuric acid in different dilutions. The results obtained have formed the subject of a separate paper [Krishna Ayar 1941]. These are briefly summarized in the following paragraphs.

(i) Each phase of the insect's life has distinct and differential requirements of temperature and humidity for its survival and development.

(ii) The adults are unable to withstand high temperatures for considerable duration. Their upper thermal death-point is about 122° F. when they are unable to stand exposure for six hours. At 113° F. it takes 48 hours to kill them. Between 113° F. and 106° F. a very much longer period of exposure is necessary to produce lethal effect. Changes of humidity do not seem to affect them at these high temperatures.

(iii) For maximal functional activity and longevity a temperature range of 90°-98° F., associated with humidity 60-80 per cent, is the optimum. The duration of life of the adults may extend to three months under these conditions. Changes in humidity also exercise great influence on the longevity of the adults, 60-80 per cent being the most favourable (Table V). At 100° F. they live for shorter periods.

TABLE V  
*Influence of humidity on the longevity of the adults*

Humidity (per cent)	Longevity of females (days)	
	Mated	Unmated
0 . . . . .	2·6	2·4
20 . . . . .	3·8	3·4
40 . . . . .	13·3	14·1
60 . . . . .	13·2	39·3
80 . . . . .	13·5	34·7
100 . . . . .	13·0	18·0

At 91° F. and 73 per cent humidity, a maximum of 91 days averaging 50·5 for 45 individuals has been recorded.

(iv) Reproduction is much reduced above 100°F., unless when the micro climate is very favourable. The egg-laying capacity decreases with rise in temperature. At 91°F. the average production per female is 46 eggs, at 93°F. 29·2, at 100°F. 25·3, at 106°F. 45 and at 113°F. 0·7 eggs, the last being in a collapsed condition.

(v) A wide range of tolerance is exhibited at each temperature with regard to humidities for oviposition. At 100°F. the optimum is between 80 and 100 per cent relative humidity, at 93°F. between 60 and 80 per cent and at 91°F. is about 70-75 per cent.

(vi) The incubation period is not much affected by variations in humidities at normal temperatures.

(vii) The upper limit of viability of egg is a little below 100°F. At 60 per cent humidity there is partial hatching, at 80 per cent there is complete hatching, but the best level for hatching and survival is 100 per cent relative humidity.

(viii) Eggs and early stage grubs are very sensitive to desiccation and high mortality is caused by changes in this factor; medium and mature grubs require medium humidities, while prepupae and pupae withstand and develop even in lower humidities like 40—20 per cent and 0 per cent. A higher humidity of 100 per cent is not conducive to the development of older stages due to fungal attack.

(ix) Mating retards longevity of the adults. At 60 per cent humidity and 93°F. the unmated male can live to the maximum period of 98 days, while a mated one lives only 58 days. Similarly, an unmated female lives longer for three months, whereas its mated sister perishes after 54 days.

(x) At favourable temperatures and humidities there is no difference in the duration of life of the different sexes but, under unfavourable conditions, the males succumb earlier.

(xi) The duration of life is much affected by food (Table VI).

TABLE VI

*Duration of life at 93°F. and 80 per cent relative humidity*

Mated or unmated	Sex	Maximum longevity without food (days)	Maximum longevity with food (days)
Unmated . . . . .	Male . . . . .	4·0	23·9
Do. . . . .	Female . . . . .	4·4	34·7
Mated . . . . .	Male . . . . .	4·0	18·5
Do. . . . .	Female not allowed to oviposit	4·6	13·2
Do. . . . .	Female allowed to oviposit	6·0	18·6

The results described in Table VI explain to a great extent why *Pempherulus* is more abundant under irrigated conditions and in more succulent varieties like Cambodia.

### Alternate food plants

An interesting phase of the investigation of the weevil is the study of its food plants other than cotton. The earlier accounts, besides being vague and conflicting, fail to provide the exact species of plants in many cases. No data on incidence, locality, susceptibility and status are available for any species. It was, therefore, evident from the outset that the host plants were imperfectly known. These studies were restricted in scope, being confined mostly to localities in Coimbatore and its environs. A few occasional collections from adjoining forests were also made. These have not only brought to light a number of hitherto unrecorded food plants, but also provide valuable information on the exact species of plants, the nature and character of infestation, the changes in the habits of the insect in relation to plant species and a series of new parasites peculiar to these changed habits. A concise summary of the data is furnished in Table VII.

TABLE VII

Name of the alternate host	Natural order	Total number examined	Average per cent of infestation	Highest per cent of infestation	Remarks
<i>Triumfetta rhomboidea</i> *	Tiliaceae	4,999	69.6	100	Doubtful host
<i>Corchorus olitorius</i>	"	1,519	27.0	51.5	
<i>Corchorus trilocularis</i>	"	684	1.2	2.8	
<i>Sida acuta</i> *	Malvaceae	5,116	16.7	80.0	
<i>Sida spinosa</i>	"	160	20.7	73.7	
<i>Sida glutinosa</i> *	"	190	12.6	91.3	
<i>Sida rhomboidea</i> *	"	278	6.5	10.0	
<i>Sida rhombifolia</i> *	"	109	4.6	50.0	
<i>Malvastrum coromandelianum</i> *	"	4,607	14.9	41.5	
<i>Hibiscus vitifolius</i> *	"	1,324	6.1	40.0	} Doubtful hosts since no live stages have been actually recovered
<i>Hibiscus ficulneus</i> *	"	740	3.0	16.6	
<i>Hibiscus esculentus</i>	"	381	14.4	30.5	
<i>Hibiscus cannabinus</i>	"	321	35.2	80.0	
<i>Urena sinuata</i> *	"	396	0.3	16.7	
<i>Hibiscus surattensis</i>	"	304	1.3	20.0	
<i>Melochia corchorifolia</i>	"	258	4.7	7.4	
<i>Abutilon hirtum</i>	"	356	10.4	52.7	
<i>Abutilon glaucum</i>	"	531	1.1	5.5	

Nine plants among these, marked with asterisk, were recorded for the first time as alternate hosts for *Pempherulus affinis*. The discovery of such a large number of plants, most of them occurring wild in nature, has set the problem of control of the pest on a different footing. It has made it clear that

the mere observance of a close period between two cotton crops will not effectively control the pest under the conditions prevailing at Coimbatore even by a prolonged close period.

Amongst the several hosts, *Triumfetta rhomboidea* is the most favoured of wild plants but adults emerging from this host do not oviposit freely on cotton. It is not clear whether this preference is due to race differences or food requirements. The importance of the discovery of unrecorded parasitic fauna in these food plants will be dealt with under parasites.

#### *Reaction of Pempherulus with reference to food*

Studies on insect dietetics are of great practical importance in affording clues for devising preventive and control measures. A series of experiments, therefore, conducted to determine the effect of different kinds of food on the fecundity and duration of life of the females of *Pempherulus* under known identical physical conditions and the results obtained have formed the subject matter of a separate paper [Krishna Ayyar, 1940, 3].

Mere supply of water does not seem to have any beneficial effect on its life-duration or reproductive powers. An exclusive carbohydrate diet produces a remarkable increase in duration of life and also, to a limited extent, fecundity. Raisin, whose composition includes a small proportion of proteins and fats, besides carbohydrates, has yielded best results. It seems to constitute an ideal food among those tested in respect of all activities, inclusive of fecundity and longevity. From an average of about four eggs without any artificial food as high an average as 76.1 eggs per female with a record number of 164 eggs as maximum per female has been obtained on a raisin diet. Results of a few tests on oviposition responses in relation to oviposition sites, such as roots, flower buds, etc. are also presented.

#### *Original home and habitat*

The investigation of the original home and primitive environment of a pest may afford the key to its present status and eventual control besides being the most promising source of efficient parasites [Myers, 1931]. Previous studies on the weevil gave no indications of the original home of the weevil. The weevil came into prominence with the introduction of an exotic variety of Cambodia cotton from Cochin China some 30 years ago. On this account as also because of its long association with this variety of cotton, it was suspected that the weevil was also imported from the same country. But a study of its geographic distribution coupled with that of its food plants and parasites largely renders this assumption open to question. It has already been noted that the genus does not extend beyond the Indo-Malayan zone. It is, therefore, evident that the immediate ancestors of *Pempherulus affinis* and its allies must have had their origin in this region. Further, it is a well-known fact that an insect in its native habitat is usually well controlled by its natural enemies. But *Pempherulus* has no effective natural enemy in cotton field and the few recorded must be regarded as of recent association since none is mentioned in previous literature. These and other considerations suggested that the pest might have commenced to infest cotton comparatively recently. The survey made in parts of Malabar district pointed that wild *bhindi* (*Hibiscus* species) should have had an older association with weevil than cotton, since the insects

preferred the former, when both were grown together. Besides, it was found to infest *Hibiscus esculentus* in localities where cotton was completely absent. Further studies revealed that the insect is found infesting wild plants in hill tracts and forests in distant parts of India, such as Malabar in the south and Dehra Dun in the north. Its association with such wild plants as *Triumfetta rhomboidea*, *Sida acuta*, *S. rhomboidea*, *Urena lobata* in virgin forests far away from any cotton cultivation would appear to be still older and more primitive than with *Hibiscus esculentus*. Among these, *T. rhomboidea* appeared to be unique in both heaviness of infestation and parasitism. With it is associated a parasitic fauna not met with in cotton tracts. These findings are highly suggestive of the possibility that *Pempherulus* is indigenous to India with some one of these wild plants (possibly *T. rhomboidea*), serving as its original or primitive habitat. Further studies in this line would prove extremely useful and interesting.

## II. STUDIES ON THE PARASITES

The investigations conducted in this line may be conveniently discussed under four main heads :—

- (a) Parasites in association with cotton
- (b) Parasites in association with food plants other than cotton
- (c) Parasites imported from other provinces
- (d) Mass-breeding experimental releases and recoveries

### PARASITES IN ASSOCIATION WITH COTTON

One predatory mite and six species of Hymenopterous parasites were obtained from *Pempherulus* during the course of the present studies. These are listed below :—

*Acarina*—*Pediculoides ventricosus* Newpt. (Tarsonemidae)

*Braconidae*—*Spathius critolaus* Nixon

*Chalcidoidea*—

*Euderus pempheriphila* Ramkr and Mani

*Eupelmus* sp.

*Aplastomorpha calandrae* (How.)

*Eupelmus urozonus* Dalm.

Unidentified Braconid (*Microbracon* sp. ?) as also a Chalcid

*Pediculoides ventricosus* Newpt. (*Acarina*—*Tarsonemidae*): It is known to have a world-wide distribution. Among its hosts may be counted the larvae, pupae and even adults of a wide range of soft-bodied insects, particularly insects of stored products and others that live in partial or complete concealment. Being an external parasite it feeds by sucking out body fluids of soft integumented insects. The life-history of the mite has been worked out by many authors [Taylor, 1937] and is well known. It has a short life and the eggs and young ones develop inside the mother and are given birth to as adults though at this stage their size is small. Soon after it commences feeding, it becomes globular in size with a small anterior projecting head. This attacks the immature stages of *Pempherulus* in the laboratory but has seldom been observed in nature in the field. Very often the grubs together with their parasites are devoured. Its utility as a means of pest control is extremely doubtful.

*Extent of total parasitism*

The course of parasitism was followed in relation to three seasonal crops sown in September and two off-seasonal crops between March and August (Fig. 4). The data are presented in Table VIII.

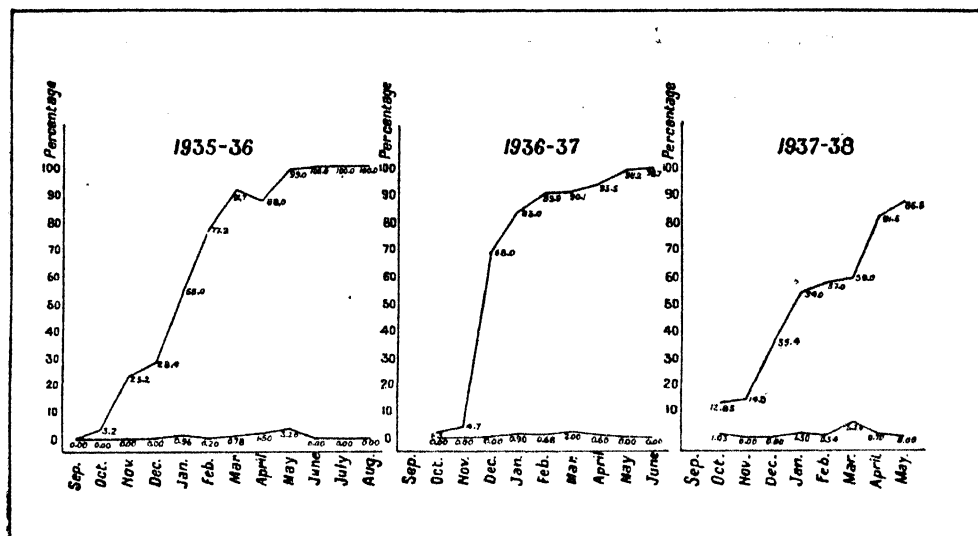


FIG. 4. Percentage infestation and parasitism in seasonal crop

TABLE VIII

*Infestation and parasitism in seasonal crops*

Month	1935-36		1936-37		1937-38	
	Percent- age of infesta- tion	Percent- age of para- sitism	Percent- age of infesta- tion	Percent- age of para- sitism	Percent- age of infesta- tion	Percent- age of para- sitism
September	..	..	..	..	..	..
October	3.2	..	1.3	..	12.85	1.03
November	23.2	..	4.7	..	14.80	..
December	28.2	..	68.0	..	35.40	..
January	55.0	1.90	83.0	0.90	54.00	..
February	77.2	0.59	89.9	0.68	57.00	0.54
March	91.8	4.70	90.1	2.00	59.00	5.20
April	88.0	1.50	93.5	0.60	..	..
May	100.0	3.20	..	..	..	..
June	100.0	..	..	..	..	..
July	100.0	..	..	..	..	..

TABLE IX

*Infestation and parasitism in off-seasonal crops*

Month	1937		1938	
	Percent- age of infesta- tion	Percent- age of para- sitism	Percent- age of infesta- tion	Percent- age of para- sitism
March	5.6	..	..	..
April	36.7	..	10.9	..
May	79.0	0.26	22.1	0.70
June	100.0	6.70	29.3	0.50
July	100.0	3.60	47.0	1.40
August	100.0	2.70	87.8	0.75
September	..	..	93.7	3.20

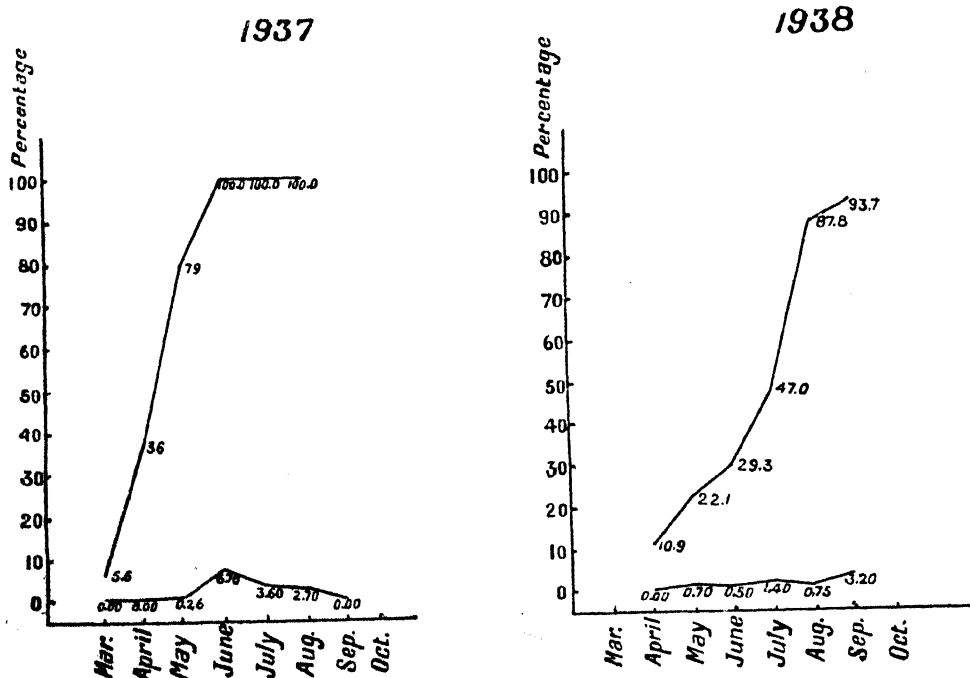


FIG. 5. Percentage incidence and parasitism in off-seasonal crops

From Table VIII it may be evident that the rate of parasitism during the three seasons shows marked irregularity which does not admit of any easy explanation. The total parasitism did not exceed 5.2 per cent in the seasonal crop and 6.7 per cent in the off-seasonal crop. Parasitism was evident in the first brood itself in the seasonal crop for the year 1937 but the peak period would appear to be February-March when second and later broods overlapped. The off-seasonal crops show a greater regularity in parasite incidence (Fig. 5).

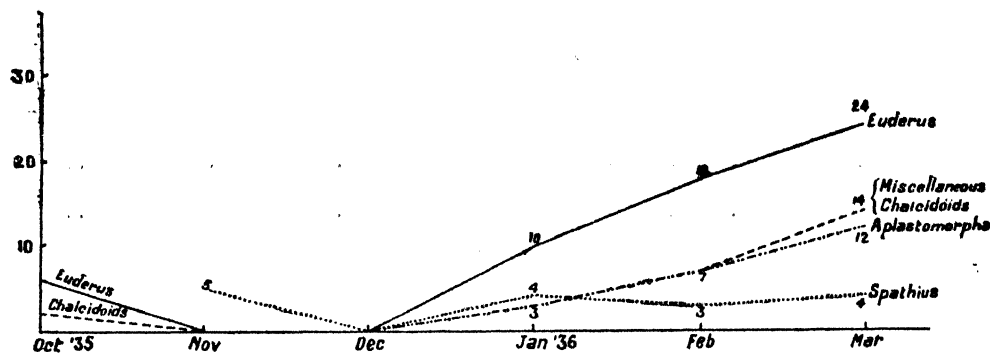


FIG. 6. Parasitism in seasonal crop, 1935-36

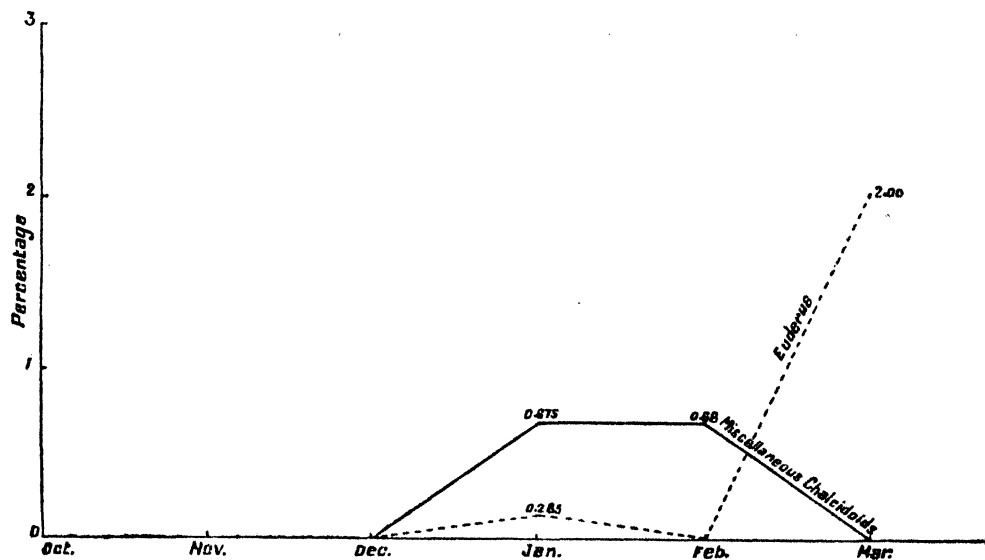


FIG. 7. Parasitism in seasonal crop, 1936-37

### Seasonal incidence of parasitism

Among parasites, *Spathius critolaus* generally appears early in the season during the first generation of the pest. Its occurrence, though in small numbers should, however, be deemed important since it happens at a time when the pest incidence is low. The importance of *Euderus pempheriphila* consists in its numerical superiority. It is comparatively abundant in January as also

in June to August. *Aplastomorpha* and *Eupelmus* sp. occur in some numbers late in the season—June to August—when the crop is to be removed. The other species were only of occasional occurrence and their role in the control of the pest may be considered to be insignificant (Figs. 6-10).

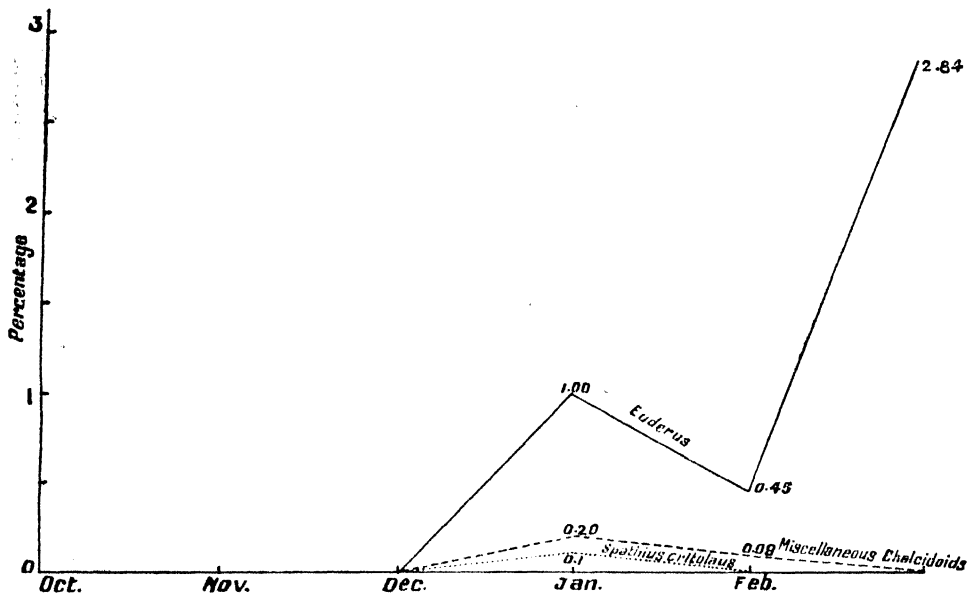


FIG. 8. Parasitism in seasonal crop, 1937-38

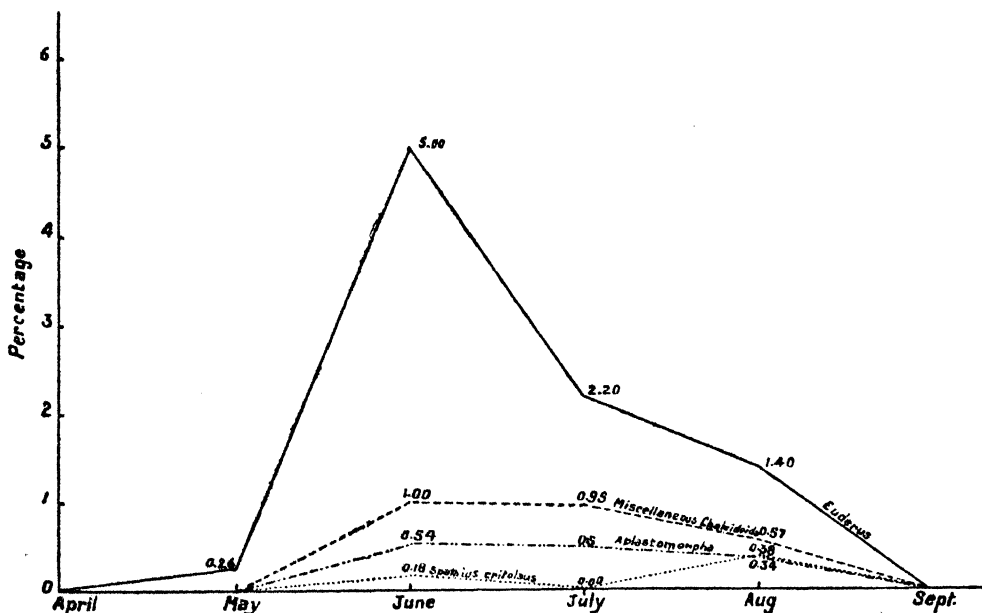


FIG. 9. Parasitism in off-seasonal crop, 1937

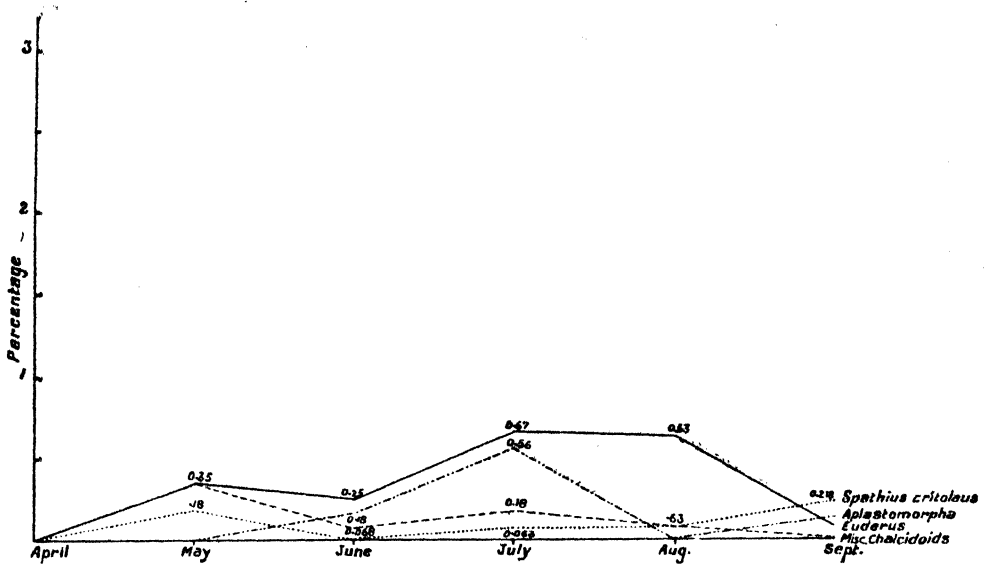


FIG. 10. Parasitism in off-seasonal crop, 1938

### Biology of the parasites

The habits and life-history of a few have been studied in detail. A detailed study of others has not been possible.

*Spathius critolaus* Nixon is a primary, ectophagous, larval parasite. A small proportion of the females is winged. Mating is not essential for oviposition. The females search out the grubs in the galleries, paralyse them by stinging and then oviposit. Only active, healthy and fairly mature grubs appear to be chosen as hosts. Generally one egg is laid on each host, although a female can lay up to a maximum of seven eggs per day, the average being two to three. The maximum number of eggs laid by a female was 53. The pre-oviposition period is normally two days. The egg stage lasts for one or two days. The larvae on hatching, feed externally on the fluid contents of the host for about four to five days. When full grown, they spin cocoons around their bodies. The prepupal and pupal periods are two or three days and eight days respectively. The adults emerge by making a circular opening first in the cocoon and later in the bark of the stem. The males generally emerge earlier (by one to three days) than the females. Parthenogenetic progeny are all males. The entire life-cycle may vary from 14 to 25 days, according to the season. The duration of life of the adult varies considerably with the nature and availability of food. When fed on the nectar of cotton flowers, they were found to live up to 5½ months. No case of hyper-parasitism has been noticed so far. A detailed account of this parasite has already been published [Krishna Ayyar, 1940,1].

The parasite has two alternate hosts : (1) the amaranthus weevil *Hypolixus truncatulus* (Curculionidae) and (2) the cotton stem-borer Bostrychid, *Sinoxy-lon sudanicum*. The discovery of these has been useful for rearing the parasites under laboratory conditions when *Pempherulus* is not available in the field. An account of their mass-breeding is furnished in a later paragraph.

*Euderus pempheriphila* Ramkr. and Mani is a dark, small-sized, Eulophid with a tiny ovipositor, attacking medium-sized grubs, found near the bark, and yet it is the most numerous amongst the weevil parasites collected from cotton fields. The female stings the host completely paralyzes it and lays in most cases a single egg, loosely and indiscriminately on any part of the host larva. Even when more than one egg is laid, it is only one that develops to maturity. The larva on hatching feeds voraciously on the host, reducing it quickly into an empty capsule. It then evacuates the meconium, turns into a short, white prepupa, soon transforms itself into a slender, naked pupa in the host-tunnel and subsequently emerges as adult. The egg-period is one day, larval period 4-7 days, pupal period 5 to 7 days and the total life-cycle varies from 12-18 days. The maximum duration of life of the adults, when fed on raisin, was 13 days. The adult is not a strong flier and is difficult to breed in captivity as seen from limited trials in the laboratory. This parasite is subject to the attack of a hyper-parasite *Eupelmella pedatoria* Ferr. in the full grown larval and pupal stages.

*Eupelmus* sp. is another primary ectophagous parasite. It lays its eggs mostly on young grubs, although it sometimes chooses more advanced, medium-sized grubs also. Egg-period is not known and larval period occupies about five days, prepupal period one day and pupal period ranges from five to nine days. It was found only occasionally in very small numbers.

*Eupelmus urozonus* Dalm. is an occasional ectophagous parasite of *Pempherulus* grubs. This species prefers full-grown host-grubs, but sometimes also attacks medium-sized ones. The egg-period occupies about one day; larval period six to eight days; prepupal period about one day; and pupal period from six to nine days averaging 7.5 days. The species also occurs in association with alternate host plants like *Sida acuta*.

*Aplastomorpha calandrac* (How.) sometimes occurs in association with alternate host plants like *Triumfetta rhomboidea*. It has been found to mate and oviposit in captivity. It also reproduces parthenogenetically giving rise to males. It lays its eggs in most cases singly after paralyzing the host-grubs, which may be either medium sized or full grown. The pre-oviposition period varies considerably up to a maximum of 22 days. Egg-period is about one day, larval period four to six days, prepupal one to three days and pupal period varies from five to ten days. The total life-cycle ranges from 16 to 17 days.

*Eupelmella pedatoria* Ferr. is a wingless, shining dark Chalcid. It parasitizes not only *Pempherulus* grubs but also those of *Hypolixus truncatulus* and *Apion corchori*. It also functions as a hyper-parasite on larva and pupa of *Euderus*. Though not economically of much significance it is of considerable scientific importance due to its peculiar habits of reproduction and its double role as a primary and secondary parasite. The life-cycle varies from 17 days in July to 23 days in November, averaging 20.7 days during the period. The duration of life of the adult ranges from 6 to 47 days averaging 19.7 days for a dozen individuals. Three generations of the parasites have been reared in the laboratory without encountering any males. Probably males are unknown in the species. A detailed account of this species has already been published [Krishna Ayyar, 1940,1].

**Other species of parasites:** An unidentified Chalcidoid. and a Braconid probably of the genus *Microbracon* have been, on rare occasions, taken from

*Pempherulus* in cotton. The Braconid has also been actually reared in the laboratory from parasitized host-grubs collected from the field.

#### PARASITES IN ASSOCIATION WITH ALTERNATE HOST PLANTS

It may be stated that as the existence of alternate host plants was itself doubted at the commencement of this scheme, parasites from this source were totally unknown. When parasites were collected from these food plants, the studies imparted a new orientation to the problem of biological control of *Pempherulus*. Nearly a thousand parasites belonging to different species were collected from *Triumfetta rhomboidea*, *Corchorus olitorius*, *Sida acuta*, *Sida glutinosa*, *Malvastrum coromandelianum* and *Hibiscus esculentus*. Among these, those secured from *Triumfetta* formed the great bulk (Tables X and XI). Particular interest has attached to the incidence of parasites since the ultimate aim of this work is to find measures for the control of the weevil. The following species have been bred from this source :—

##### Chalcidoidea—

1. *Entedon pempheridis* Ferr.
2. *Dinarmus sauteri* Masi\*
3. *Eupelmus urozonus* Dalm.
4. *Euderus pempheriphila* Ramkr. and Mani

##### Braconidae—

5. *Spathius labdacus* Nixon
6. *Spathius critolaus* Nixon
7. *Rhaconotus cleantes* Nixon
8. *Rhaconotus menippus* Nixon

Besides these, a Nematode parasite, *Geomermis indica* Steiner has also been noted. Five of these species, namely, *Entedon pempheridis*, *Dinarmus sauteri*, *Spathius labdacus*, *Rhaconotus cleantes* and *Rh. menippus* are absent in cotton fields. Most of the species are new to science and have only been recently described.

#### Seasonal incidence

The data collected on this aspect are presented in Tables X and XI. Although the parasites occur throughout the year, their maximum incidence seems to be from September to November, which period synchronizes with the early stages of the first brood of *Pempherulus*. If any of these can establish in cotton, it may be able to keep the pest under control. In fact a single collection of two species of these parasites was actually made from cotton fields, when the infested alternate food plants were spread in the cotton crop.

#### Biology of the parasites

It has not been possible to study the life-histories of all the species. A separate account, embodying all available information has already been published [Krishna Ayyar, 1940,1], brief summaries of which are furnished below :—

*Entedon pempheridis* Ferr : This species is totally absent in cotton fields, although it is the most widely distributed and most numerous among alternate

\* *Dinarmus coimbatorensis* Ferr., recorded in previous papers is a synonym of *D. sauteri* Masi.

TABLE X

Seasonal incidence of parasites in nature from alternate host plants based on data collected every month from different localities

Parasites	1937-38										1938						Per- cent- age of each kind				
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Per- cent- age of each kind								
													Apr.	May	June	July		Aug.	Sept.	Oct.	
<i>Eutelon pemph- ridae</i>	1	...	1	1	23	103	73	51	23	4	13	21	56.8	4	27	30	33	27	18	43	42.4
<i>Spathius labda- cus</i>	2	...	...	...	...	16	26	24	32	3	7	23	24.0	...	20	34	18	11	26	38	34.2
<i>Dinarmus sau- teri</i>	1	...	...	16	8	8	16	11	3	...	7	2	13.0	2	12	13	14	9	8	10	15.8
<i>Rhaconotus clean- thes</i>	...	...	...	...	...	...	10	1	...	...	...	...	2.0	...	5	5	8	...	3	4	5.8
<i>Spathius crilo- lus</i>	...	...	...	...	2	3	2	...	1	3	2	2	2.7	...	...	...	...	1	...	...	0.3
<i>Euderus pem- pheriphila</i>	...	...	1	...	...	...	1	...	...	...	...	...	0.35	...	...	1	...	...	...	...	0.3
<i>Aplatomorpha calandrac</i>	...	...	...	...	1	...	...	...	...	...	1	...	0.35	...	...	1	...	...	...	...	0.3
<i>Eupelmus uro- zonae</i>	...	...	...	...	...	...	...	...	...	...	1	...	0.2	...	...	...	...	...	...	...	...
Doubtful cases	...	...	...	...	...	...	2 Br.	...	...	1	...	...	0.6	...	...	1	...	...	...	2	0.9
Total	4	...	2	17	34	130 (A)	130 (B)	87 (C)	59	11	31	48	100.0	6	64	85	73	48	55	97	100.0

(A) *Spathius criolus* from *Melochia* excluded, (B) 1st record of Nematode parasite, (C) 2nd record of Nematode parasite

TABLE XI  
*Alternate host plants—consolidated summary, 1936-38*

Plant species	1936					1937					1938				
	Total No. of plants examined	Percent- age of infesta- tion	Average percent- age of parasitism	Total parasites recovered	Total No. of plants examined	Percent- age of infesta- tion	Average percent- age of parasitism	Total parasites recovered	Total No. of plants examined	Percent- age of infesta- tion	Average percent- age of parasitism	Total parasites recovered	Total No. of plants examined	Percent- age of infesta- tion	Average percent- age of parasitism
<i>Triumfetta rhomboidea</i>	...	...	...	...	2,362	55.8	8.9	423	2,637	81.9	12.0	415	2,637	81.9	12.0
<i>Sida acuta</i>	1,772	9.5	...	...	2,632	14.5	3.3	18	692	43.5	5.1	15	692	43.5	5.1
<i>Sida spinosa</i>	93	24.7	...	...	42	22.9	19.0	8	25	...	...	...	25	...	...
<i>Melastrom coromandelianum</i>	1,432	20.7	...	...	1,529	14.0	1.8	2	1,346	9.9	...	...	1,346	9.9	...
<i>Corchorus olitorius</i>	143	14.7	...	...	878	34.0	2.9	14	498	18.3	...	...	498	18.3	...
<i>Melochia corchorifera</i>	...	...	...	...	163	7.4	8.0	1	95	...	...	...	95	...	...

host-plant parasites. It has been bred from *Pempherulus* infesting *Triumfetta rhomboidea*, *Sida acuta*, *Corchorus olitorius*, *Malvastrum coromandelianum*, also from *Apion* grubs boring into *C. olitorius*. It is a primary larval parasite and is the only endophagous species so far noted on *Pempherulus*. A single egg is probably laid inside the host-grub and the larva on hatching consumes the entire contents of the host. Just prior to pupation it issues out of the empty host and pupates in the tunnel. Since parasitism by this species is the highest, it is likely to prove very useful.

*Dinarmus sauleri* Masi : It is a primary ectophagous parasite of *Pempherulus* grubs. Its life-cycle covers a period of 17-21 days. It has been collected from *Pempherulus* attacking *T. rhomboidea*, *Sida acuta*, *Corchorus olitorius* and *Hibiscus esculentus*. It is absent in cotton fields.

*Spathius labdacus* Nixon : It is a large, spotted-winged species, with a long ovipositor. It has been bred only in association with one wild food plant, namely, *T. rhomboidea*. It is a primary, ectophagous parasite, choosing its victims from among the healthy non-parasitized full-grown grubs. Its life-cycle ranges from 18 to 22 days. It has been successfully reared in the laboratory. Parthenogenetic reproduction is common.

*Rhaconotus cleantes* Nixon and *R. menippus* Nixon : These slender Braconids are more or less similar in appearance and habits. They have been bred in association with *T. rhomboidea*, *C. olitorius* and *Sida acuta*. These are also primary and ectophagous. These attack full-grown host-grubs. The life-cycle roughly occupies 16 to 24 days. These parasites are entirely absent from cotton fields.

Other species of parasites such as *Euderus pempheriphila*, *Eupelmus urozonus*, *Spathius critolaus*, etc. have already been reviewed under cotton field-parasites.

*Nematode parasite* (*Geomermis indica* Steiner) : This generally infests mature grubs boring into *T. rhomboidea* and feeds on the internal fluids. It has been identified by Dr Steiner as *Geomermis indica*. The genus itself is known only from U. S. A. so far.

#### IMPORTED PARASITES

Over 20 different species of parasites numbering in all about 600 individuals consisting mostly of Braconids and Chalcidoids obtained from 30 different lots of infested plant material were collected in the course of the north Indian tour. The entire collection comprised parasites either from Curculionid stem-borers or other stem-borers belonging to allied families. Attempts were made to breed them on *Pempherulus* grubs. Among these, only two species were seen to possess possibilities of utilization. One of these is a species of *Spathius* parasitizing *Dinoderus* in bamboo, collected from Jawalapur (United Provinces) and the other, a larger Braconid—*Doryctes* sp.—attacking Cerambycids (undetermined) boring into *Millettia* at Dehra Dun.

*Spathius vulnificus* Wlkn. from Jawalapur : This is a winged form of *Spathius*, closely resembling the local species (*S. critolaus* Nixon) in size and general appearance. Being a larval parasite, its potential efficiency is great. It searches out and follows up the grubs of *Pempherulus* and *Hypolixus*, supplied to them in stems in cages. Its fecundity is 89 eggs. As many as 20 eggs have been seen to be deposited on a single grub of *Hypolixus*, which is capable of supporting all these to maturity. This heavy super-parasitism

though an apparent advantage for mass-breeding, is wasteful in the field. The life-history has been worked out. The total life-cycle varied from 19 to 29 days, made up of an egg-period of two days, larval period of six days, prepupal period of six days and a pupal period varying from 6 to 13 days. The males emerge one or two days earlier than females. The pre-oviposition period ranged from 4 to 20 days. The duration of life extended up to three months. Since it admitted of rearing in the laboratory, mass-breeding was attempted. It was more or less encouraging in the beginning. It, however, showed a tendency to a gradual decline in numbers from generation to generation. It may be easier to obtain thousands of these parasites by importing parasitized material from the original source where it is plentifully available.

*Doryctes* sp. from *Millettia* : This species is a comparatively large-sized Braconid which freely develops on *Hypolixus* and *Pempherulus* grubs provided inside stems in cages. It is capable of laying a maximum of 27 eggs. The total life-cycle occupies about 24 days with an egg-period of two days, larval period of six to seven days, prepupal period of about two days and a pupal period of 11—13 days. The pre-oviposition period varies from 4 to 21 days. The egg-laying capacity is comparatively poor. Mass-breeding was attempted but without much success.

#### MASS-BREEDING, EXPERIMENTAL RELEASES AND RECOVERIES

The main attempt at mass-breeding centred round *Spathius critolaus*. The imported species, *Spathius vulnificus*, also received some attention.

*Spathius critolaus* : Availability of host material is one of the chief factors in mass multiplication. *Pempherulus* stages are only available during the cotton season and even then only in small quantities ; during the rest of the year it breeds on two alternate hosts.

*Hypolixus truncatulus* and *Sinoxylon sudanicum* : The former host was utilized for rearing parasites inside the laboratory in small cages and the latter was useful for breeding in large out-door cages. The Bostrychid—*Sinoxylon*—generally bores into wilting and wilted Cambodia cotton stalks ; occasionally it also bores into living plants in the field. The female tunnels into the stem and constructs an enlarged chamber for pairing, into which the male enters. After mating, the female makes a new side tunnel, where it deposits the eggs. The grubs, on hatching, make long galleries along the stem and pupate in the galleries filled with wood-dust and excreta. The adult beetles emerge by boring their way out of the stems. The entire life-cycle occupies roughly six to seven weeks. The adults can be easily collected during the afternoons, especially in November and December. There appear to be four distinct broods. The mature grubs of this borer form the preferred hosts of the parasite. In the case of *Hypolixus*, eggs are laid in cavities hollowed out in stems which are later on sealed. The average egg-laying capacity is about 55·4 eggs per female, with the maximum rising up to 78. It passes through five larval instars before turning into a prepupa. The total life-cycle averaged 42·7 days within a range of 35—55 days. The egg-period averages about 4·6 days, the larval 24 days and the prepupal and pupal together about 15 days. The duration of life of the adult female averaged 42·6 days and that of male 38·5 days. The study of this host has an added significance in the present studies. It is a heavily parasitized insect in nature with a set of over

14 different species of parasites. As many as five or six species among these are also parasitic on *Pempherulus* in cotton.

By a judicious handling of these two hosts about 9000 parasites were actually reared during the period. Table XII furnishes data on their numbers and seasonal occurrence.

TABLE XII  
*Spathius critolaus* (1936-1938)

Month	Collections from outdoor cage			Rearing in laboratory cage		
	1936	1937	1938	1936	1937	1938
January . . . . .	..	76	39	..	..	..
February . . . . .	..	55	..	..	26	10
March . . . . .	274	53	81	..	32	19
April . . . . .	185	90	116	60	16	1
May . . . . .	7	288	101	355	10	13
June . . . . .	9	182	295	62	31	..
July . . . . .	264	159	710	19	11	..
August . . . . .	385	117	873	5	30	25
September . . . . .	279	301	565	180	29	50
October . . . . .	685	324	541	183	8	65
November . . . . .	365	232	..	20	..	..
December . . . . .	179	260	..	33	9	..
Total . . . . .	2,632	2,137	3,321	917	202	183

It may be noticed from Table XII that the emergence of the parasites was greatest during July to October, which is a very convenient time for releasing them in the fields to control the first generation of the pest. The sex-ratio of the parasite shows a slight preponderance of females averaging 53 per cent from Bostrychid hosts and 58 per cent from *Hypolixus*. About 5-6 per cent of these are winged forms.

#### Releases

The release of this species was attempted twice in the field cages during the period. The first of these was vitiated by the following unforeseen cause and therefore could not be pursued. The plants grown in cages for the purpose

during the off-season were heavily covered by aphids and coccids with swarms of attendant ants. The ants had their nests so thickly honey-combed in and around these cages that their control was found impossible. The parasites liberated were destroyed by the ants before they could settle down on plants for parasitization. A second trial was made in another cage. Though this proved better, it suffered from another type of unexpected handicap. The plants being grown in a field cage had to be artificially infested with weevils. The weevils were not available for the purpose during October due to the absence of any infested cotton crop in the vicinity. Therefore, adults bred from alternate host plants like *Triumfetta* were introduced into the cage for infestation. It was found later that such adults (though of the same identical species) did not readily take to cotton; and only very poor oviposition had taken place with the consequent scarcity of suitable grub stages in the plants at the time of parasite releases. Pest infestation and parasite releases were, however, carried on continually for some more time. All plants that indicated weevil attack externally were pulled out and examined for parasite. The results obtained, despite the handicap, were of sufficient significance.

TABLE XIII  
*Parasite releases and recoveries*

Month	No. of plants examined	Percentage of plants attacked	Percentage of parasitism based on		Number of parasites liberated	Remarks
			Pest stage	Total infestation		
November 1937 .	12	91.7	58.3	38.9	121	Live and dead stages were only 5 in all
December 1937 .	12	91.7	20.0	6.3	48	
January 1938 .	14	100.0	21.4	17.6	23	
February 1938 .	6	100.0	60.0	33.3	4	
Total .	44	95.9	31.5	22.1	196	Average percentage of infestation and parasitism

The data recorded above show that the percentage of parasitism as per live and dead stages varied from 20—60 per cent with an average of 31.5 per cent for the entire lot. The live stages were rare due to light infestation. This level of recovery has to be deemed encouraging in the case of a stem-borer. It is clear that provided adequate releases are made at the

proper time under favourable conditions, the parasite will work efficiently. These experiments, however, call for more trials in view of the many points in favour of this parasite. Its life-cycle is only a fourth of the period taken by the host and it can, therefore, complete not less than three generations by the time the host completes one. It is not wasteful in egg-laying and it chooses only healthy grubs. The average egg-laying capacity of the parasite (22-24) is nearly equal to that of the host (about 24 eggs). The sex-ratios are nearly equal which will enable the parasite easily to overtake the pest. It has no hyper-parasite and can tide over off-seasons by living on the two alternate hosts. Besides, it can by itself live long in nature. It occurs in most localities where the pest is found and at a critical time when the first brood of the pest is developing. The low parasitism of 1 per cent recorded under field conditions has to be ascribed to a multiplicity of factors. A certain proportion of hosts is always inaccessible due to their concealed habits. This is overcome to some extent by the presence of a long ovipositor in the parasite. Another is the choice of victims being restricted to medium and mature host-grubs and it may be that only a fraction of the grubs are sufficiently far advanced for their acceptance. This is partly got over by the prolonged grub period and the uneven development of the hosts which makes the host-grubs available almost throughout the season. Again, the host-grubs lie scattered in different plants located at distances. They can be reached by the small proportion of winged forms. It can also be remedied artificially by large releases. Notwithstanding these advantages it is possible that its biotic potential may be low under field conditions.

The imported species, *Spathius vulnificus*, was, as stated already, amenable to rearing in the laboratory. At one time a fair number of adults was available and a small number (about 77 consisting of 71 females) was liberated in a cotton field but no recoveries could be made.

### III. PRESENT POSITION OF THE PROBLEM AND CONCLUSIONS

The present investigation, covering a short period of three years, has not reached a stage when the possibilities or otherwise of controlling *Pemphorus* by natural enemies can be definitely declared. Its biology, geographical distribution, alternate food plants, original home and natural enemies have been studied. The first infestation of cotton is due to weevils, emerging from its weed hosts and wild food plants near cultivated areas. It is noted that the insect has only recently become a major pest of cotton in south India which it probably began to attack about 25—30 years ago. Previous to this, the weevil probably confined its attack to wild food plants like *Triumfetta rhomboidea*, *Sida acuta*, etc. and its numbers are supposed to have been controlled by the action of the set of parasites associated with them in this wild habitat. The introduction and extensive cultivation of Cambodia cotton and its continued expansion in areas and intensity resulting in vast 'monocultures' provided the weevil with a new domain, having an inexhaustible supply of food. Its conditioning in this variety gradually adapted it for attacking other varieties, such as country cottons. The weevil's habits have also adjusted themselves to the altered environment. The parasites of the weevil in its natural and wild habitat failed to accompany the same successfully into the

cotton fields. But the abundance of weevils in cotton may not be entirely due to the absence of parasites. A partial study of the physical ecology of the weevil suggests that extremes of climatic conditions in south India are not sufficiently great to offer an effective check on its multiplication as in other parts of India. It looks as if a series of years of severe drought may cause a serious reduction in the population of the weevil by the partial destruction of its wild host plants. On the other hand, a series of seasons with heavy rainfall would appear to produce more favourable conditions for its increase.

Experiments on pests like stem-borers necessarily require a good many years before definite results emerge. A considerable volume of precise knowledge on these aspects has, however, been accumulated now but these studies have only covered the essential preliminary stages. Many vital aspects on the biology of the pest still await investigation. The studies of alternate food plants of the weevil have reached a stage when the main problem of their attraction and nutrition can be proceeded with. A further study of the parasites discovered in association with cotton and other food plants has to be made. The exact relation of the few known enemies to the pest and their individual rôles in the host-parasite complexes call for further study. Particular attention may be directed to two lines of investigation which seem to be of special interest. Mass-multiplication of parasites associated with cotton and their liberation at suitable times may effect some measure of control. The second is the colonization of parasites (associated with alternate host plants) in cotton fields. The percentage of parasitism in nature is low but it is important since this small force of parasitic element is an essential factor in maintaining an equilibrium in nature. It is reasonable to suppose that conservation, multiplication, and liberation of parasites in fields, will at least act as an auxiliary agent in pest-control.

#### ACKNOWLEDGEMENTS

In conclusion, the writer takes this opportunity to record his grateful thanks to the Indian Central Cotton Committee for financing the scheme and the Cotton Specialist and the members of his section, who have afforded him help in one form or another in carrying out the investigation during the period. He also wishes to place on record the enthusiastic co-operation he has always received from the Assistants and Fieldmen, who have been associated with him during the three years of the scheme. He is indebted to Sir Guy A. K. Marshall and the specialists of the British Museum for the identification of weevils and parasites.

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# ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY

## VIII. THE ACID CHARACTER OF HYDROGEN CLAY IN RELATION TO SOME PROBLEMS OF SOIL SCIENCE\*

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**I**N parts V—VII of this series [Mitra, 1936 ; Mitra, Mukherjee and Bagchi, 1940 ; Mitra, 1940] several features of the acid character of hydrogen clays have been discussed. Parts V and VI mainly dealt with variations of the total neutralizable acid, that is, the base exchange capacity, under different conditions, and the characteristics of the titration curves have been discussed in part VII. The following aspects of the relation of the acid character of hydrogen clay with some problems of soil science are discussed in this part :—

1. Regular, specific and mixed cation effects in the interaction of hydrogen clay with neutral salts and bases.
2. The liberation of aluminium from hydrogen clay by neutral salts.
3. The role of cation effects in the estimation of the base exchange capacity of hydrogen clays and soils.
4. Variations in the form of the titration curves of entire hydrogen clay and hydrogen bentonite fractions of several Indian soils and bentonites†.
5. Variations in the properties of sub-fractions of the entire hydrogen clay fraction of a soil.
6. Alterations in the properties of hydrogen clay on the removal of free inorganic oxides contained in it.

\* The results given in this paper have been taken from the published **Annual Reports** for 1934-35, 1935-36, 1936-37, 1937-38, and 1938-39 on the working of a scheme of research into the ' Properties of Colloid Soil Constituents ' financed by the Imperial Council of Agricultural Research, India.

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† Bentonites are formed by the weathering of volcanic rocks and have chemical composition similar to that of soil. They are known to contain clay minerals belonging to the montmorillonite group and thus form an important link in the chain of systems which lie between simple substances such as  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$  and standard clay minerals on the one hand and the very complex hydrogen clays on the other.

## EXPERIMENTAL

The method of preparation of the hydrogen clays and hydrogen bentonites and experimental procedure have been described in part IV [Mukherjee *et al.*, 1936], and parts V and VII. Particulars regarding the soils and bentonites used and the hydrogen clays and hydrogen bentonites which were obtained from them are given in Table I.

TABLE I  
*Particulars regarding the soils and bentonites used*

Lab. No.	Description of soil or bentonite*	Silica-sesquioxide ratio (molar) of entire clay fraction	Reference number of corresponding hydrogen clay or hydrogen bentonite
13	Brownish yellow soil (unmanured) from Government Farm, Suri (Bengal) collected at a depth of 6-12 inches from Agricultural Chemist's experimental plot, block A 1-16, plots Nos. 3, 5, 16	2.34	E
14	Highland acid soil from Government Farm, Burdwan (Bengal) collected at a depth of 0-6 inches from block B, plot No. 40 of the Farm	1.94	F
20	Neutral calcareous soil (brown loam) from Government Seed Farm, Kalyanpore (U. P.) collected at a depth of 0-6 inches	2.10	H
25	Black cotton soil (neutral, calcareous) from Satara (Bombay), collected at a depth of 0-6 inches	2.50	I
32	Neutral black soil from Bilaspur near Raipur (C. P.), collected at a depth of 0-6 inches	2.54	K
22	Red lateritic soil (acidic) from Government Farm, Dacca (Bengal) collected at a depth of 0-6 inches	1.99	L
34	Black soil from Government Farm, Akola (Berar) collected at a depth of 0-9 inches	2.19	M
33	Bhata red laterite soil from C. P. collected at a depth of 0-9 inches	1.88	N
46	Non-lateritic calcareous soil (B-type) from Government Farm, Padegaon (Nira, Poona) collected at a depth of 0-12 inches	2.51	Padegaon-B
51	Acid soil from Government Farm, Jorhat (Assam), collected at a depth of 0-6 inches	2.58	Jorhat-F
53	Highland acid soil on old alluvium from Government Farm at Latekujan (Assam), collected at a depth of 0-6 inches	2.47	Latekujan-F
B. O. C. 1	Bentonite from Hati-Ki-Dhani . . . . .	2.86	Hati-Ki-Dhani-B
B. O. C. 3	Bentonite from Bhadres . . . . .	2.90	Bhadres-B

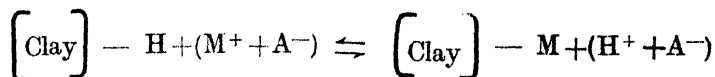
\* The samples of bentonite were kindly supplied by the Assam Oil Company.

## RESULTS

1. *Regular, specific and mixed cation effects in the interaction of hydrogen clay with neutral salts and bases\**

In parts V and VI [Mitra, 1936 ; Mitra *et al.* 1940] it has been shown that the total neutralizable acid of a hydrogen clay sol, called in agricultural science the base exchange capacity (b. e. c.), is, unlike acids in true solution, not a fixed quantity but depends on cation effects, the pH and, in some cases, on the time allowed for the interaction with the base. The higher the pH the larger is the b. e. c. The cation effects are illustrated by : (a) the dependence of the b.e.c. calculated at the inflexion point and, more strikingly, at a fixed pH (e.g. pH 7.0), on the cation of the base ; (b) the much larger b. e. c. obtained on titration in the presence of, or on leaching by, neutral salts than with the base alone ; and (c) the differences observed between the effects of various cations of neutral salts. In the absence of salts the b. e. c. decreases in the order  $\text{Ca(OH)}_2 > \text{Ba(OH)}_2 > \text{NaOH}$  which, however, changes to  $\text{Ba(OH)}_2 > \text{Ca(OH)}_2 > \text{NaOH}$  in the presence of a fixed concentration of the corresponding chlorides. The differences in the relative effect of  $\text{Ba}^{++}$  and  $\text{Ca}^{++}$  ions have been traced to the fact that in the presence of  $\text{BaCl}_2$  or  $\text{CaCl}_2$  the greater part of the reaction with the base usually takes place between pH 3.5 and 5.5 ; whereas, when no salt is present, the reaction is mainly confined within the range of pH 5.5.-6.5. In the presence of the salts the cation effect is regular in the sense that it follows the lyotrope series and is determined by the order of electrical adsorption of cations together with their hydration envelopes [Mukherjee, 1922]. At the comparatively speaking higher pH values which obtain in the absence of the salts, the cations are probably adsorbed in a dehydrated state which would account for what has been called the irregular cation effect operating under these conditions.

The mixture of the sol and salt contains  $\text{H}^+$  ions some of which are present in the intermicellary liquid and others† are associated with the colloidal particles or flocs. The incomplete displacement of the  $\text{H}^+$  ions by the cations of the salt is to be referred to the balanced nature of the reaction which can be represented according to the following simple scheme ignoring complicating factors :



where  $\text{M}^+$  and  $\text{A}^-$  are respectively the cation and the anion of the added salt. The intensity of the back reaction is determined by the total concentration of  $\text{H}^+$  ions in the liquid. When a hydrogen clay is repeatedly leached with the salt solution, the  $\text{H}^+$  ion concentration of the salt extract rapidly decreases as the leaching proceeds, thus favouring more and more the direct reaction. This process is also very much enhanced if the salt solution is a buffer having a high pH. In the interaction with a base the back reaction is almost absent, thus securing a more complete replacement of the  $\text{H}^+$  ions by the cations.

\* The work described in this section was carried out by Mitra along with others. Some results have been given in part VI.

† Aluminium ions appear to be present in the double layer in addition to hydrogen ions (see sub-section 2). Apart from this, the nature of the primarily adsorbed anions and the crystal structure of the particles require to be considered in detail.

Other peculiarities of cation effects, not discussed in the previous parts of this series and not clearly recognized by previous workers, have also been recorded in this paper. They are observed when the added salt has cations other than those of the base used for the titration. Such mixed cation effects are of interest. The soil absorption complex usually contains more than one type of exchangeable cations and the part they may play, individually and relatively to each other, on the base exchange and other reactions of the complex is not definitely known. Ionic antagonism effects are well known in colloidal behaviour [Freundlich and Scholz, 1922 ; Mukherjee and Ghosh, 1924]. A study of the mixed cation effects may also throw light on observations such as that of Renold [1936] who found that a mixed permutite, e.g. a K-Ba-permutite prepared from a Ba-permutite by treatment with a K-salt, has not the same base exchange property as another having an identical composition and amount of exchangeable  $Ba^{++}$  and  $K^+$  ions but prepared from a K-permutite by interaction with a Ba-salt.

The cation and the pH effects are illustrated below.

TABLE II

*Base exchange capacity in m. e. base per 100 gm. of oven-dried hydrogen clay using NaOH, Ba (OH)<sub>2</sub>, and Ca (OH)<sub>2</sub>*

System	NaOH		Ba(OH) <sub>2</sub>		Ca(OH) <sub>2</sub>	
	At inflex. pt.	At pH 7.0	At inflex. pt.	At pH 7.0	At inflex. pt.	At pH 7.0
Sol E . . . . .	2.2 (5.4)*	15.4	20.6 (6.0)	25.0	21.5 (5.8)	26.2
Sol E + 0. 1N BaCl <sub>2</sub> . . . . .	...	...	28.0 (4.6)	> 42.4	...	...
Sol E + 0. 1N CaCl <sub>2</sub> . . . . .	...	...	...	...	21.2 (4.4)	40.6
Sol E + 0. 1N NaCl . . . . .	16.1 (5.0)	26.4	...	...	...	...
Sol Padegaon B . . . . .	57.0 (7.4)	53.5	89.0 (8.05)	74.0	91.0 (8.02)	80.0
Sol Padegaon-B + 0.002 N NaCl . . . . .	70.0 (8.0)	63.0	78.0 (8.0)	67.0	80.0 (8.0)	69.0
Sol Padegaon-B + 0.002 N BaCl <sub>2</sub> . . . . .	55.0 (5.7)	68.0	67.0 (6.1)	70.0	63.0 (6.1)	70.0
Sol Padegaon-B + 0.002 N CaCl <sub>2</sub> . . . . .	52.0 (5.63)	67.0	67.0 (5.66)	70.0	60.0 (6.05)	69.0
Sol Padegaon-B + 0.10 N NaCl . . . . .	85.0 (7.53)	80.0	90.0 (7.2)	87.0	90.0 (7.46)	85.0
Sol Padegaon-B + 0.10 N BaCl <sub>2</sub> . . . . .	65.0 (5.26)	114.0	74.0 (5.0)	122.0	70.0 (5.1)	116.5
Sol Padegaon-B + 0.10 N CaCl <sub>2</sub> . . . . .	63.5 (5.30)	110.0	72.5 (5.15)	120.0	70.0 (5.1)	116.5

\* The figures in brackets denote the pH at the inflexion point of the titration curve.

The variations of the b. e. c. recorded in Table II are summarized below :

Experiment	Variations of b. e. c. observed	Inference
1. Sol titrated with different bases	$\text{Ca(OH)}_2 > \text{Ba(OH)}_2 > \text{NaOH}$	Irregular, or specific cation effect
2. Mixture of sol and salt titrated with the corresponding base	<p>(a) <math>\text{Ba(OH)}_2 &gt; \text{Ca(OH)}_2 &gt; \text{NaOH}</math> at inflexion point of E and at pH 7.0 of both E and Padegaon-B</p> <p>(b) <math>\text{NaOH} &gt; \text{Ba(OH)}_2 &gt; \text{Ca(OH)}_2</math> at inflexion point of titration curves of Padegaon-B</p> <p>(c) The inflexion point gives a smaller b. e. c. of Padegaon-B and <math>\text{BaCl}_2</math> (or <math>\text{CaCl}_2</math>) mixture compared with Padegaon-B itself</p>	<p>Regular cation effect</p> <p>The apparent order <math>\text{Na}^+ &gt; \text{Ba}^{++} &gt; \text{Ca}^{++}</math> is to be referred to the much higher pH at inflexion point in the titration curve of the mixture of sol and NaCl compared with the mixture containing <math>\text{BaCl}_2</math> (or <math>\text{CaCl}_2</math>). The pH effect masks the cation effect.</p> <p>The regular cation effect is masked by the pH effect as the inflexion point in the titration curve of the mixture occurs at a much lower pH than that of the sol</p>
3. Mixture of Padegaon-B and a fixed conc. of NaCl, $\text{BaCl}_2$ or $\text{CaCl}_2$ titrated with different bases	$\text{Ba(OH)}_2 > \text{Ca(OH)}_2 > \text{NaOH}$	The regular cation effect. The smaller b. e. c. of the mixture of sol and 0.1 N $\text{BaCl}_2$ or $\text{CaCl}_2$ obtained on titration with NaOH than with $\text{Ba(OH)}_2$ or $\text{Ca(OH)}_2$ indicates some sort of an ionic antagonism between the comparatively few $\text{Na}^+$ ions of the NaOH and the large number of $\text{Ba}^{++}$ or $\text{Ca}^{++}$ ions of the salt. The strong adsorption of the $\text{Ba}^{++}$ and $\text{Ca}^{++}$ ions and their capacity to displace $\text{H}^+$ ions from the double layer appear to be somewhat inhibited by $\text{Na}^+$ ions present at a much lower concentration
4. Mixture of Padegaon-B and a fixed conc. of different salts titrated with the same base	<p>(a) <math>\text{BaCl}_2 &gt; \text{CaCl}_2 &gt; \text{NaCl}</math> at pH 7.0</p> <p>(b) <math>\text{NaCl} &gt; \text{BaCl}_2 &gt; \text{CaCl}_2</math> at the inflexion point of the titration curve</p>	<p>Regular cation effect</p> <p>Regular cation effect is masked by the pH effect</p>

## 2. The liberation of aluminium from hydrogen clay by neutral salts†

Aluminium ions are known to be set free by the interaction of neutral salts with acid soils and hydrogen clays [Paver and Marshall, 1934]. There is some difference of opinion regarding the nature of the reaction by which the aluminium is liberated. A direct exchange of the  $\text{Al}^{+++}$  ions by the cations of the added salt has been suggested by some workers [Daikuhara, 1914; Kappen, 1916] while others [Page, 1926; Wilson, 1929] consider that aluminium is brought into solution by a secondary dissolution of aluminium oxide by the acid generated on the addition of a salt. An exchange of both  $\text{H}^+$  and  $\text{Al}^{+++}$  ions by the cations of the salt has also been postulated [Paver and Marshall, 1934].

The possible sources of these displaced  $\text{Al}^{+++}$  ions are: (i) free  $\text{Al}_2\text{O}_3$  contained in the hydrogen clay, (ii)  $\text{Al}^{+++}$  ions inside the lattice of the mineral constituents of the clay and (iii)  $\text{Al}^{+++}$  ions present on the surface in (a) a primarily or (b) secondarily adsorbed condition. Aluminium in all these three forms may react with acids and bases. Toxic properties of acid soils have often been attributed to aluminium found in the soil solution. There is evidence to show that  $\text{Al}^{+++}$  ions are stable on the surface of colloidal particles of aluminium oxide sols at a pH as high as 6.0 [Mukherjee *et al.*, 1932; also unpublished work of B. Majumdar in this laboratory].

In part VII of this series it has been shown that at concentrations below 0.002*N* alkali metal cations liberate practically no aluminium from hydrogen clays and consequently an exchange of  $\text{H}^+$  ions against the cations of the salt has to be postulated to account for the titratable acid of the neutral salt extract. The subject has been studied in detail by one of us (B. Chatterjee). While a detailed account will be published separately, some of his results are given below.

Increasing amounts of  $\text{BaCl}_2$  were added to hydrogen clay H. Table III illustrates the relations between (i) the amounts of Al liberated, (ii) the total acidity of the clear supernatant liquid above the coagulum of the sol and salt mixture obtained on centrifuging this mixture in resistance glass containers, and (iii) the amount of Ba adsorbed by the hydrogen clay.

TABLE III

*Relation between the Al liberated, the total acidity of the supernatant liquid and the Ba adsorbed by the hydrogen clay*

(50 c. c. of the sol taken for each experiment; time of interaction 24 hours)

Concentration of added $\text{BaCl}_2$	m. c. per 100 gm. colloid		
	Displaced Al	Displaced acid	Adsorbed Ba
0.01 <i>N</i>	3.1	11.4	11.1
0.02 <i>N</i>	4.9	11.9	12.7
0.04 <i>N</i>	8.2	14.4	15.2
0.09 <i>N</i>	12.6	17.0	18.5
1.00 <i>N</i>	22.0	22.0	24.0

† The work discussed in this section was carried out by Chatterjee with the help of others. Details will be published in a separate series of papers.

As the concentration of the salt increases more Al is liberated and at a concentration of  $1.0\text{ }N$ , the liberated Al, the adsorbed Ba and the total acidity all have almost identical values. A fair agreement between the total acid and the quantity of Ba adsorbed is observed at all concentrations of the added  $\text{BaCl}_2$ . It appears from the results that an exchange of both  $\text{H}^+$  and  $\text{Al}^{+++}$  ions for the  $\text{Ba}^{++}$  ions takes place. With increasing salt concentration more  $\text{Al}^{+++}$  ions are exchanged and the exchange of the two ions does not seem to be independent, although at very low concentrations of the salt only  $\text{H}^+$  ions are exchanged.

The view that  $\text{Al}^{+++}$  ions are liberated by direct exchange and not following a secondary dissolution is in harmony with the fact that the curves (Fig. 1) obtained on plotting (a) m. e. of Al liberated, (b) the free and total acids of the clear supernatant liquid and (c) the amount of Ba adsorbed, against the concentration of the added  $\text{BaCl}_2$ , all have the form of the usual adsorption isotherm.

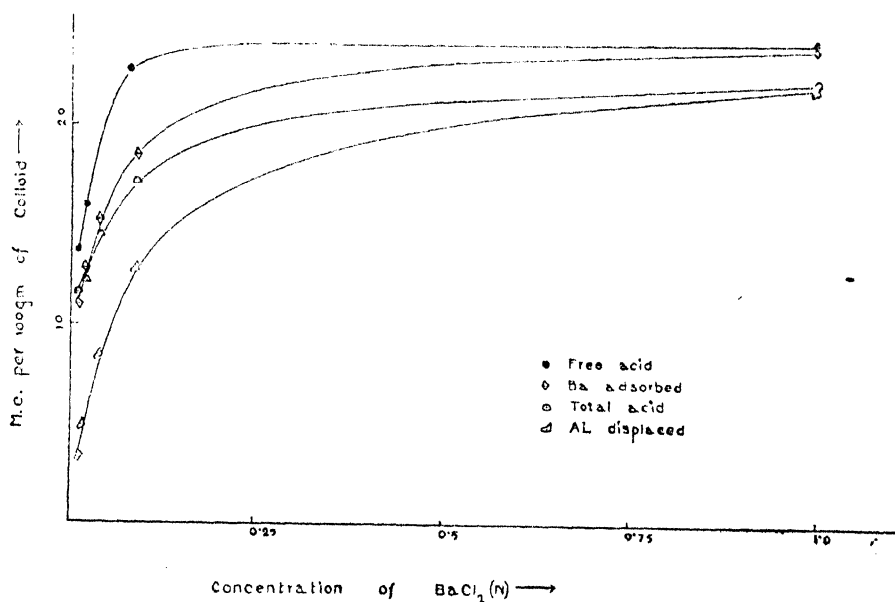


FIG. 1 Curves showing the relation between the Al liberated, the free and total acids of the supernatant liquid and the amount of Ba adsorbed

The above view is further supported by the following results obtained with Padegaon-B which show that the amount of aluminium liberated is materially the same, both when the  $\text{pH}$  of the sol is allowed to decrease on the addition of the salt as also when the  $\text{pH}$  is kept constant by the use of a suitable buffer.

If the aluminium found in the supernatant liquid were dissolved from the hydrogen clay by the acid set free, larger quantities would have been found when the buffer was not used. At constant  $\text{pH}$  also the amount of displaced Al steadily increases with the concentration of  $\text{BaCl}_2$ .

TABLE IV

*Al liberated from hydrogen clay with and without the addition of buffer*

With buffer*			Without buffer		
Conc. of salt	pH	M. e. Al displaced per 100 gm.	Conc. of BaCl <sub>2</sub>	pH	M. el A displaced per 100 gm.
0.10 N BaCl <sub>2</sub> + 0.016 N Na-Ac.	3.60	20.0	0.10 N	2.57	20.9
1.0 N BaCl <sub>2</sub> + 0.02N Na-Ac.	3.64	40.9	1.0 N	2.54	40.9

\* Sodium acetate + acetic acid.

### 3. *The role of cation effects in the estimation of the base exchange capacity of hydrogen clays and soils\**

The base exchange capacity of a soil is an extremely ill-defined quantity [Hissink, 1935] and concordant results are seldom obtained by different routine methods used for estimating it [Crowther and Martin, 1925]. The uncertainty mainly arises from the difficulty of an unequivocal definition of the amount of reactive or exchangeable hydrogen (and aluminium). The variations of this quantity are capable of being accounted for by cation effects formulated by us and the equilibrium pH of the solution. The time of attainment of equilibrium is also of importance, especially in these systems where interaction in interfaces or inner surfaces [Wiegner, 1935] are involved. It follows from theoretical considerations [Mukherjee *et al.*, 1925] that if the concentration of cations is high the relative differences observed between them should become smaller. When the pH is high, its effect may even override the cation effect as previously shown. In other words, with a sufficiently high concentration of cations and of hydroxyl ions the difference in the b. e. c. obtained using different salts should be less and a definite limiting value would be obtained indicating the total amount of reactive hydrogen and aluminium ions which are probably present at different affinity levels and remain associated with the particles of the hydrogen clay. It is assumed that additional complicating factors, such as the dissolution of the particles and exposure of inner layers, are absent. A comparative study of some routine methods of estimating the b. e. c. has been made and the results including those published previously [Mitra and Mitra, 1940] are given in Table V.

Parker's [1929] and Schollenberger's [1930] methods give nearly the same b. e. c. At pH 7.0 and normal concentration, the difference between NH<sub>4</sub><sup>+</sup> and Ba<sup>++</sup> ions vanishes. In the titration in presence of N BaCl<sub>2</sub> up to pH 7.0 the pH and cation effects are comparable to what obtain in the above two methods and the values obtained by these three methods mutually agree. Schofield's

\* The work discussed in this section has been carried out by Mitra and Mukherjee (S. K.) with the help of others and details are being published in a separate series of papers. Part I of this series has appeared [Mitra and Mitra, 1940].

method [1933] gives nearly the same result for Latekujan F but about 6 per cent higher value for the other hydrogen clay. More marked differences have been observed in the case of soils.\*

TABLE V

*B. e. c. in m. e. per 100 gm. of oven-dried (105°C.) hydrogen clay obtained by different methods*

Hydrogen clay	Titration† with baryta in presence of $N$ $BaCl_2$	Parker's†† method	Schollenberger's‡ method	Schofield's‡‡ method	Estimation of cations adsorbed on leaching with neutral normal solutions of		
					$N_4HCl$	$BaCl_2$	$CaCl_2$
Jorhat-F . . . . .	33.0	33.0	32.0	35.0	34.0	22.0	20.5
Latekujan-F . . . . .	56.5	54.0	55.0	55.0	56.0	42.0	40.0

† To mixtures of the hydrogen clay and  $N$   $BaCl_2$  contained in a series of Jena glass bottles increasing amounts of  $Ba(OH)_2$  are added; the mixtures are thoroughly shaken and kept overnight; the pH is measured on the following day and the b. e. c. is calculated from the inflexion point of the titration curve.

††  $Ba$  adsorbed on leaching with neutral normal solution of barium acetate is displaced on further leaching with a neutral normal solution of  $NH_4Cl$  and estimated.

‡  $NH_4$  adsorbed on leaching with neutral normal solution of ammonium acetate is estimated.

‡‡ The amount of lime taken up from a half-neutralized solution (pH 7.1) of p-nitrophenol with this base is estimated.

The somewhat higher value obtained by Schofield's method can be traced to the following factors:—

(a) in this method the equilibrium pH is somewhat higher (7.1) and, what is more important, the system is always maintained at this pH whereas in Parker's and Schollenberger's methods it has been found that a considerable portion of the total leaching solution used has a pH near about 6.4 after it has percolated through the hydrogen clay or soil; the pH rises slowly to 7.0 after the leaching has been continued for some time;

(b) a longer time\*\* (16-18 hrs) of interaction is allowed in Schofield's method than in the other methods where the leaching is usually finished within 6 hrs;

(c) the greater adsorption of  $Ca^{++}$  ions compared to  $Ba^{++}$  and  $NH_4^+$  ions near about pH 7.0 in agreement with the irregular cation effect.

$Ba^{++}$  and  $Ca^{++}$  ions are adsorbed from their neutral normal solutions in amounts which are smaller than the b. e. c. determined by the methods of Parker, Schollenberger and Schofield (Table V). The amount of  $NH_4$  adsorbed from a neutral normal solution of  $NH_4Cl$ , however, is in fair agreement with this b. e. c. In the methods of Parker and Schollenberger the acetates act as a buffer so that leaching proceeds near about pH 7.0. When barium or calcium chloride is used the pH of the medium has been found to be near about 6.4, i.e. below 7.0 even after leaching with 500 c. c. of the solution, which accounts for the smaller amounts of  $Ba$  and  $Ca$  adsorbed from their chlorides. Using  $NH_4Cl$ , however, the pH rises up to 6.8 and this is partially, if not wholly, responsible for the apparently greater effect of the monovalent  $NH_4^+$  ions.

\* Unpublished work of Mr S. K. Mukherjee.

\*\* It has been found by Schofield [1933] that on allowing a still longer time of interaction a somewhat higher value is obtained.

4. *Variations in the form of the titration curves of the entire hydrogen clay and hydrogen bentonite fraction of several Indian soils and bentonites†*

The more general features of the titration curves of hydrogen clays prepared from the entire clay fractions have been discussed in part VII of this series and are briefly stated below: The different strong bases give titration curves having different forms. The potentiometric titration curves with caustic alkalis indicate a weak monobasic acid character (discussed below) with an inflexion point which lies between  $pH$ 's 7.2 and 8.5. The alkaline earth hydroxides, on the other hand, give curves resembling those of a strong monobasic acid. The strong or weak acid character, however, is only apparent and the titration curves reveal several features not ordinarily expected with acids in true solution. For example, the potentiometric and conductometric titration curves with a given base offer entirely conflicting evidence regarding the strength of the acid. In contrast to the weak acid character of the potentiometric caustic alkali curves the corresponding conductometric curves show a sharp minimum indicative of a strong acid. On the other hand, the alkaline earth hydroxides give a conductometric curve with a flat rounded minimum suggesting that a weak acid is being titrated, while as stated above the corresponding potentiometric curve resembles that of a strong acid. In part VII, these features, difficult to understand from the classical electrochemical standpoint, have been reconciled in the light of the theory of the electrical double layer and of adsorption of ions as postulated by one of us [Mukherjee, 1921, 1922]. Apart from these and other features of the titration curves which are common to the hydrogen clays we have studied, there are features which are different for different hydrogen clays. Reference to some of these has been made in part VII. These differences are very likely to be useful in the characterization and classification of the soils [Anderson and Byers, 1936] and are more fully discussed below. An error may easily be made in forming any conclusion regarding the acid character of hydrogen clays in general in the absence of observations on a sufficiently large number of them prepared from soils of widely different origin and type. Moreover, the properties of the entire clay fraction is an integral of those of the particles of different sizes of which it is composed and a study of the sub-fractions should be of great help in the classification of soils. Our work on the sub-fractions has been discussed in the next section.

Figs. 2, 3, 4 and 5 illustrate the different types of potentiometric titration curves. The curves given in the figures were obtained on titrating hydrogen clays Latekujan-F, Padegaon-B, F, M, N and K and the hydrogen bentonites Hati-Ki-Dhani-B and Bhadres-B.

The NaOH curves are of three different types:

(a) To the first type belong the titration curves of the hydrogen clay F and the hydrogen bentonite Hati-Ki-Dhani-B given in Fig. 2. The curves resemble those of a dibasic acid. They have an initial strong acid character and a weak inflexion in the acid region. In these respects there is a resemblance with silicic acid sols [Chatterjee, 1939]. Further studies regarding the significance of this dibasic character are under way. The second inflexion occurs in the neutral to weakly alkaline region. Silicic acid sols do not show it in this region of  $pH$ .

† This work was carried out by Mitra.

(b) The second type of dibasic NaOH curves is illustrated by those of Latekujan-F also shown in Fig. 2. They have an initial weak acid character. Similar types of curves were obtained on titrating a hydrogen kaolinite\* prepared from a sample of the mineral from Singbhum (Bengal).

(c) To the third type of NaOH curves given in Fig. 3. belong those of the majority of hydrogen clays studied by us. The titration curves given in this figure are those of hydrogen clays Padegaon-B and N and the hydrogen bentonite Bhadres-B. The curves resemble those of a weak monobasic acid.

The  $\text{SiO}_2 : \text{R}_2 \text{O}_3$  ratio of the hydrogen clays showing the first two types of curves are 2.47 and 1.94. The hydrogen clays showing the third type of curves have  $\text{SiO}_2 : \text{R}_2 \text{O}_3$  ratios 1.88 and 2.51. It is apparent that this ratio which represents the mass chemical composition does not determine the form of the titration curve.

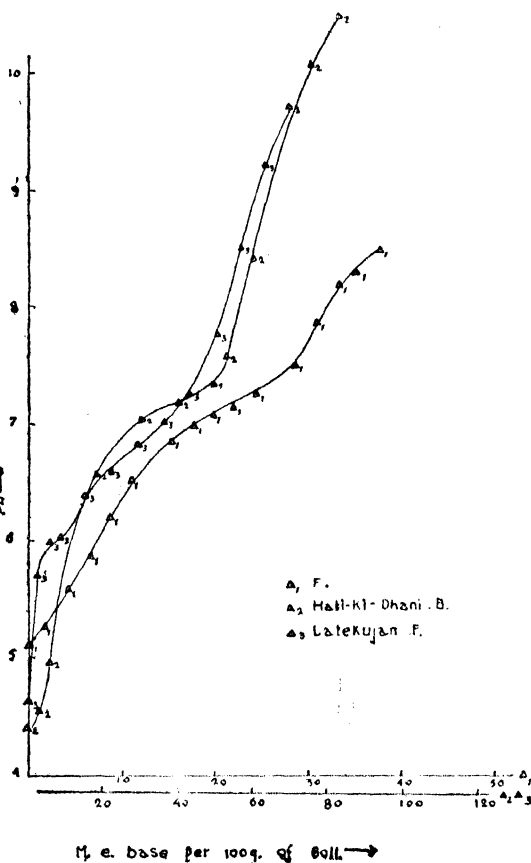


FIG. 2. Potentiometric titration curves of entire hydrogen clay and hydrogen bentonite fractions having a dibasic acid character

The  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  curves given in Figs. 4 and 5 are of the following types :

(a) N and Bhadres-B show an initial rise followed by a buffering characteristic of weak acids.

(b) The second group shows a comparatively flat initial run followed by a more or less sharp inflexion given by strong acids (Padegaon-B and K). The majority of the hydrogen clays studied by us show titration curves of this type. Differences are observed in the sharpness of the inflexion point and its location in the pH scale. The inflexion point usually lies between pH's 5.5 and 6.3 and, in a few cases, between pH's 6.3 and 7.0.

(c) Hati-Ki-Dhani-B shows a strong dibasic acid character which has not been observed by us so far with any hydrogen clay when titrated with alkaline earth hydroxides.

\* Unpublished work of Mitra.

(d) The fourth group shows a definite lowering of pH in the initial stages of the titration, e.g. the titration curves of M. This peculiar feature for which a simple explanation is difficult to suggest has also been observed in the titration curves of sub-fractions of M.

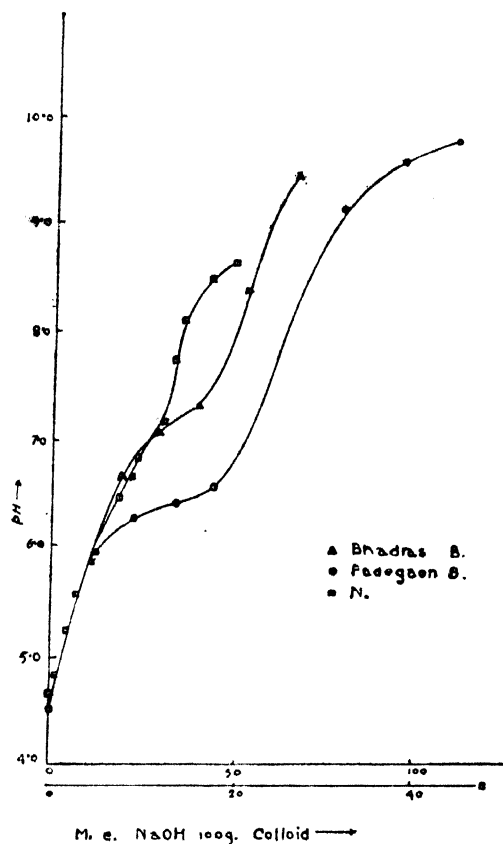


FIG. 3 Potentiometric titration curves with NaOH of entire hydrogen clay and hydrogen bentonite fractions having a weak monobasic acid character

These observations are of a novel nature and are worth following up in detail. The complexities we have observed compel the conclusion that our current notions about the acid character of clays and soils and mineralogical and X-ray analyses independent of electrochemical studies are not adequate for scientific purposes.

##### 5. Variations in the properties of sub-fractions of the entire hydrogen clay fraction of a soil\*

The entire clay fraction consists of soil particles of all sizes below  $2\ \mu$  and according to recent work the fractions containing particles of different sizes do not always have the same chemical and mineralogical composition or

\* This work has been carried out by Mitra. Details will be given in a separate series of papers

base exchange capacity [Marshall, 1935]. The identification of the mineral constituents of the sub-fractions is of considerable interest and several well-known physical methods, e.g. X-ray, thermal and optical analyses have been requisitioned for this purpose. Valuable information may be obtained through the application of the electrochemical technique including a comparison of the inflexion points and forms of the titration curves of the different fractions and their base exchange capacities calculated per gramme ( $T_g$ ) and per sq. cm. ( $T_s$ ) of the external surface. Its importance has not so far been recognized. Its usefulness may be further increased by similar studies of standard clay minerals.\* About 40 sub-fractions of typical Indian soils have been examined by us with this object in view. The relation between the particle size and the electrochemical properties of colloidal solutions is also of considerable theoretical interest.

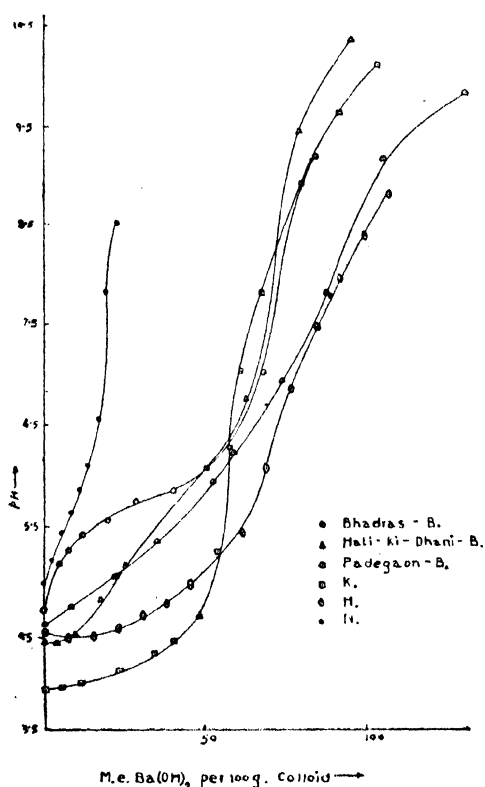


FIG. 4 Different types of potentiometric titration curves with  $\text{Ba}(\text{OH})_2$  of entire hydrogen clay and hydrogen bentonite fractions

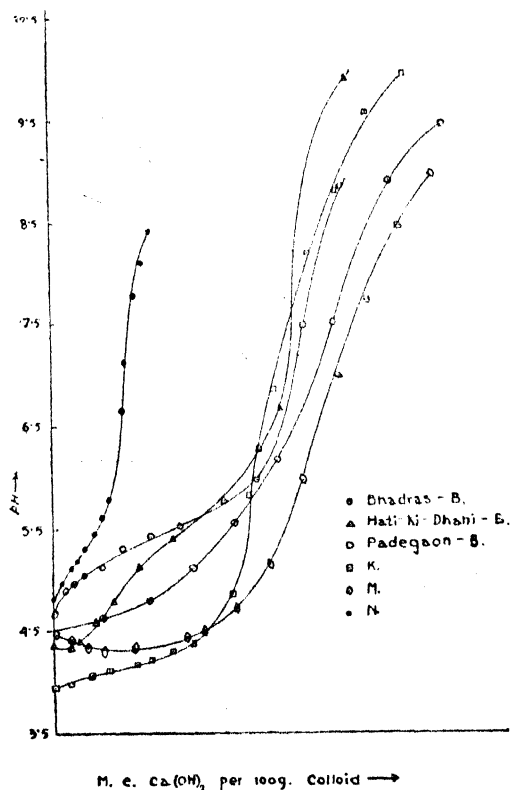


FIG. 5 Different types of potentiometric titration curves with  $\text{Ca}(\text{OH})_2$  of entire hydrogen clay and hydrogen bentonite fractions

The particle sizes, chemical compositions and b. e. c.'s ( $T_g$  and  $T_s$ ) calculated from the titration curves with  $\text{NaOH}$  of six sub-fractions of the entire hydrogen clay fraction of the black cotton soil from Padegaon have been given in Table VI, and the titration curves of four of them in Fig. 6.

\* The properties of clay minerals are being studied by Mitra.

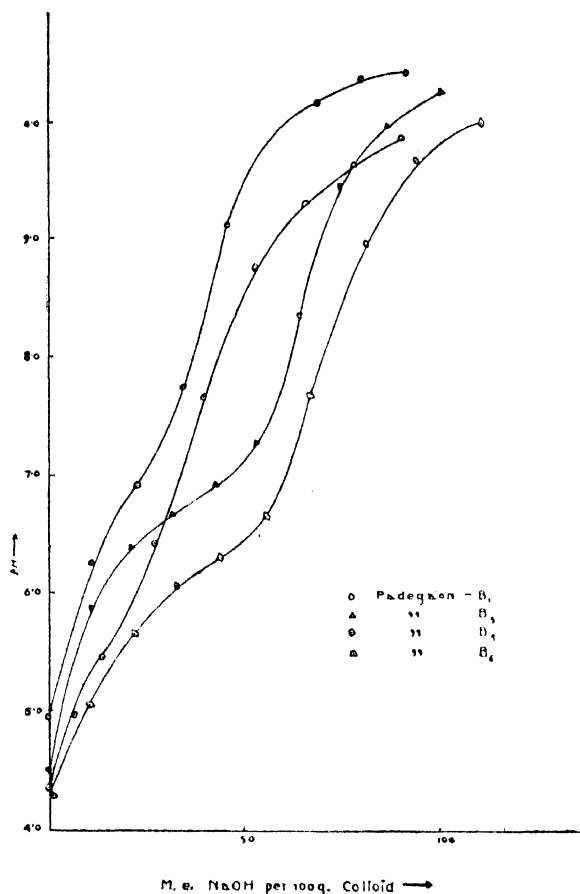


FIG. 6. Potentiometric titration curves with NaOH of sub-fractions of hydrogen clay isolated from the Padegaon soil

TABLE VI

*Chemical composition and base exchange capacity of sub-fractions of hydrogen clay isolated from the Padegaon soil*

Reference No. of sub-fraction	Mean equivalent spherical microns	Chemical composition on the ignited basis			Base exchange capacity	
		SiO <sub>2</sub> per cent	Al <sub>2</sub> O <sub>3</sub> per cent	Fe <sub>2</sub> O <sub>3</sub> per cent	M. e. per 100 gm (T <sub>g</sub> )	M. e. per sq. cm. of surface × 10 <sup>7</sup> (T <sub>s</sub> )
1	1.1	59.3	19.8	12.8	46.5	230.0
2	0.15	56.3	21.7	17.0	59.5	40.0
3	0.07	59.5	21.6	14.5	63.0	20.0
4	0.03	64.5	18.0	11.4	63.0	9.6
5	0.018	66.7	17.5	11.0	70.0	5.5
6	<0.015	60.0	26.8	10.5	40.0	<2.3

The sub-fractions were obtained by graded centrifugalization—a Sharples supercentrifuge was used—of the entire clay following Ayre's procedure as described by Whitt and Bayer [1937]. In calculating the particle size and the external surface the same density of the different fractions and a spherical symmetry of the particles were assumed. The variations in properties of the sub-fractions with diminishing particle size may be summed up as follows :—

Property	Variations
1. Chemical composition—	
(a) Percentage of $\text{SiO}_2$ . . . . .	Increases ignoring fractions 1 & 6
(b) Percentage of $\text{Al}_2\text{O}_3$ . . . . .	Decreases ignoring fractions 1 & 6
(c) Percentage of $\text{Fe}_2\text{O}_3$ . . . . .	Decreases ignoring fraction 1
2. Base exchange capacity—	
(a) $\text{Tg}$ . . . . .	Increases except for fraction 6 which has the smallest $\text{Tg}$
(b) $\text{T}^a$ . . . . .	Decreases
3. Form of titration curves . . . . .	No marked variations with the possible exception of fraction 6

The variations in chemical composition may arise from (a) isomorphous replacements [Marshall, 1935 ; Hendricks *et al.*, 1930] within the lattice of the constituent minerals, (b) differences in relative proportions of several types of clay minerals and/or inert materials, e.g. 'free' silica and sesquioxides.

The fact that the different fractions give more or less the same type of titration curves with the possible exception of fraction 6 indicates that they contain essentially the same acid material. The isomorphous replacements mentioned above would probably give rise to appreciable differences in the features of the curves.

The variations in  $\text{T}_s$  may be referred, at least in part, to differences in the chemical composition. Fractions 4 and 5, however, have nearly the same composition and the variations of  $\text{T}_s$  in their case do not admit of such an explanation. The increase in  $\text{T}_s$  of these fractions with the particle size signifies that the reaction with the base is not confined to the external surface alone but fresh layers are continuously exposed as the action with the base proceeds and/or the particles have considerable internal surfaces where the reaction takes place.

#### 6. Alterations in the properties of hydrogen clay on the removal of free inorganic oxides contained in it\*

The inorganic colloidal material of soil is associated with varying amounts of 'free' oxides of Si, Al and Fe. A comparative study has been undertaken of the changes in (i) the chemical composition, (ii) the nature of titration curves with bases, and (iii) the b.e.c.'s calculated from them consequent on treatments aiming at the removal of these free oxides. The methods developed by previous investigators are not free from the criticism that they may

\*This work has been carried out by Mitra along with others. Details will be published in a separate series of papers.

decompose or alter the nature of the exchange complex proper and may not effect a complete separation of the free oxides. It is of interest to compare the changes brought about by them. Results\* given below illustrate these changes.

The hydrogen clay L from the red lateritic soil from Dacca was treated by the method of Truog *et al.* [1936]. The b. e. c.'s calculated at the inflexion points of the titration curves of L and its derivative  $L_d$  obtained after the treatment have been given in Table VIII and the results of fusion analysis in Table VII.

TABLE VII

*Chemical composition of the hydrogen clay from the Dacca lateritic soil before and after removal of free inorganic oxides*

Hydrogen clay	Chemical composition on the ignited basis		
	SiO <sub>2</sub> per cent	Al <sub>2</sub> O <sub>3</sub> per cent	Fe <sub>2</sub> O <sub>3</sub> per cent
L	51·2	36·0	12·0
$L_d$	57·5	38·0	5·7

TABLE VIII

*B. e. c. of hydrogen clay from Dacca lateritic soil before and after removal of free inorganic oxides*

Hydrogen clay	B. e. c. in m. e. base at inflexion point of titration curve with		
	NaOH	Ba(OH) <sub>2</sub>	Ca(OH) <sub>2</sub>
L	16·3 (8·2)	17·5 (7·1)	19·0 (6·8)
$L_d$	24·5 (9·5)	23·5 (9·0)	25·0 (9·1)

The figures in brackets denote the *pH* values at the inflexion points. The b. e. c.'s of  $L_d$  have been calculated at the second inflexion point (see below).

The alterations in properties consequent on the removal of the free oxides are very significant. The b. e. c. of  $L_d$  is definitely greater than that of L and shows that inert materials having negligible b. e. c. have been removed. The chemical composition of  $L_d$  approaches that (SiO<sub>2</sub>—55·45 per cent; Al<sub>2</sub>O<sub>3</sub>—45·5 per cent) of kaolinite if allowance is made for some isomorphous replacement of Al by Fe. The titration curves of  $L_d$  (Fig. 7)

\*Obtained with the help of Sankarananda Mukherjee.

with all three bases have the same form and reveal a weak dibasic acid character, a feature also shown by kaolinite\*\*. The titration curves with the three bases of L, on the other hand, have markedly different forms\*\*\*. Further, the b. e. c. of  $L_d$  at the second inflexion point is very nearly equal to that of kaolinite. The observations on the whole, indicate that kaolinite is the dominant mineral constituent of the clay fraction of the Dacca laterite soil.

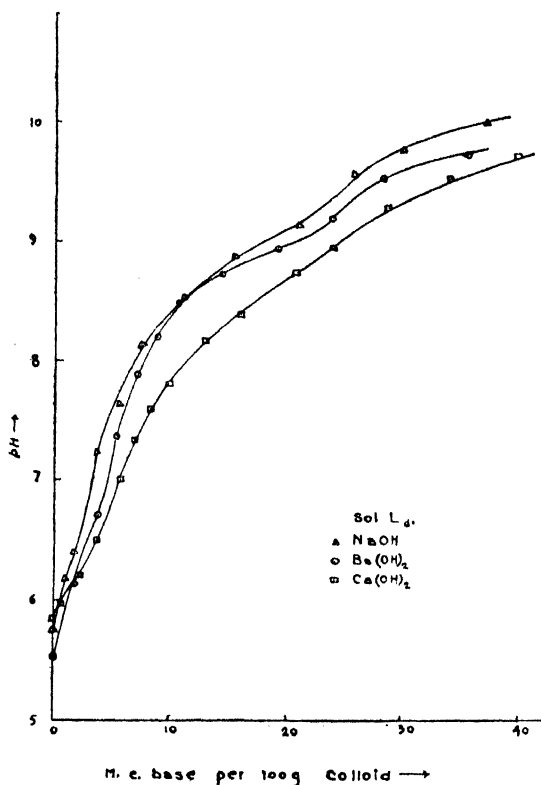


FIG. 7. Potentiometric titration curves of hydrogen clay from Dacca lateritic soils after removal of free inorganic oxides

### SUMMARY

1. The base exchange capacity (b. e. c.) of a hydrogen clay is not a fixed quantity but depends on the pH and cation effects and in some cases on the time allowed for the interaction with the base. The higher the pH the larger is the b. e. c. The cation effects are illustrated by (a) the dependence of the b. e. c. calculated at a fixed pH on the cation of the base used for the titration, (b) the much larger b.e.c. obtained on titration in the presence of neutral salts than with the base alone and (c) the differences observed between the effects of various cations of neutral salts. In the absence of salts, the

\*\*Unpublished work of Mitra (R. P.) and Mitra (D. K.).

\*\*\*They show the following features: NaOH curve: weak monobasic;  $Ba(OH)_2$  and  $Ca(OH)_2$  curves: strong monobasic. (See section 4).

b.e.c. follows the order  $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{NaOH}$  which illustrates an irregular or specific cation effect in that the relative effects of the  $\text{Ca}^{++}$  and  $\text{Ba}^{++}$  ions are in violation of the lyotrope series. In the presence of a fixed concentration of the corresponding chlorides the order changes to  $\text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2 > \text{NaOH}$  and the cation effect is regular. The difference between the relative effects of the  $\text{Ca}^{++}$  and  $\text{Ba}^{++}$  ions in the two cases has been traced to the fact that in the presence of the salts the greater part of the interaction with the base takes place at a much lower  $p\text{H}$ , usually between 3.5 and 5.5, than when no salt is added. In the latter case, the reaction is mainly confined within the range of  $p\text{H}$  5.5 to 6.5.

2. The b. e. c. of several hydrogen clays has been estimated by the methods of Parker, Schollenberger, Schofield and by titration with  $\text{Ba}(\text{OH})_2$  in the presence of  $N \text{ BaCl}_2$  and the results discussed in the light of the  $p\text{H}$  and cation effects.

3. Both  $\text{H}^+$  and  $\text{Al}^{+++}$  ions are exchanged for the cations of a neutral salt on interaction with a hydrogen clay. With increasing salt concentration more and more  $\text{Al}^{+++}$  ions are exchanged although at very low concentrations of the salt only  $\text{H}^+$  ions are exchanged. The quantity of  $\text{Al}$  exchanged on the addition of a fixed concentration of the salt is materially the same both when the  $p\text{H}$  decreases on the addition of the salt as also when it is kept constant by the use of a suitable buffer.

4. Differences have been observed in the form of the titration curves of hydrogen clays prepared from the entire clay fractions of several Indian soils and their importance in the classification and characterization of the soils discussed. The  $\text{NaOH}$  curves are of three types: weak monobasic, which is most common; weak dibasic; and strong dibasic. The  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  curves are of four types each: strong monobasic, the most common type; strong dibasic; weak monobasic; and strong monobasic but showing an actual lowering of the  $p\text{H}$  on the addition of the base in the initial stages of the titration.

5. Hydrogen clays prepared from six sub-fractions of the entire clay fraction of an Indian black cotton soil give nearly the same type of titration curves. With diminishing particle size, the base exchange capacity calculated per gramme increases except for the finest fraction but calculated per sq. cm. of the external surface, the b.e.c. diminishes.

6. Marked alterations in the base exchange capacity, chemical composition and form of titration curves of a hydrogen clay prepared from the entire clay fraction of an Indian laterite soil have been observed consequent on the removal of its free silica and sesquioxides by the method of Truog and coworkers.

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# INTERACTION BETWEEN HYDROGEN CLAYS AND NEUTRAL SALTS

## I. THE NATURE OF THE INTERACTION RESPONSIBLE FOR THE LIBERATION OF ALUMINIUM\*

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WHEN a neutral salt is added to a hydrogen clay or an acid soil, an acid reaction is developed and the neutral salt extract often contains Al and Fe. There is no unanimity of opinion regarding the mechanism by which Al and Fe are brought into solution. Two theories have been put forward to explain the nature of the reaction. The one, advocated by Daikuhara [1914] and Kappen and coworkers [1916, 1921, 1926, 1929], suggests that Al and Fe are liberated as the result of a simple exchange of these ions by the cations of the added salt. The acid developed has been ascribed to the normal hydrolysis of the resulting Al- and Fe-salts. The other theory supported by Page [1926], Magistad [1925], Kelly and Brown [1926] and Mattson [1933] assumes that in this reaction the main replacement is one of  $H^+$  ions by the cation of the added salt. The free acid thus formed dissolves aluminium and iron oxides contained in the soil or clay. Paver and Marshall [1934] have recently investigated the interaction between neutral salts and hydrogen clays. They consider that a direct exchange of both  $H^+$  and  $Al^{+++}$  ions by the cations of the added salts takes place and they have postulated that a hydrogen clay is really a mixed clay, viz. H-Al-clay.

The methods for the preparation and purification of hydrogen clays and the general experimental arrangements used in this work for the estimation of neutralizable acid and the amounts of  $Ba^{++}$  adsorbed were the same as described in previous publications from this laboratory [Mitra, 1936; Mukherjee, *et al.*, 1937; Mitra, *et al.*, 1940; Mitra, 1940]. The amount of Al present in the neutral salt extract was estimated by means of 8-oxyquinoline using the method of Berg [1927]. The free sesquioxides were removed by the methods of Tamm [1922] and Truog, *et al.* [1936].

\*The results given in this paper have been taken from the published Annual Reports for 1938-39 and 1939-40 on the working of a 'Scheme of Research into the Properties of Colloid Soil Constituents' financed by the Imperial Council of Agricultural Research, India

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The following soils were used for this work :

Description of soil	Silica-sesquioxide ratio (molar) of entire clay fraction	Reference number of corresponding hydrogen clay
Neutral calcareous soil from Govt. Seed Farm, Kalyanpore (U. P.) collected at a depth of 0 — 6 in.	2·10	H
Red lateritic soil (acidic) from Govt. Farm at Dacca (Bengal) collected at a depth of 0 — 6 in.	1·99	L
Non-lateritic calcareous soil (B-type) from Govt. Farm at Padegaon (Nira, Poona) collected at a depth of 0 — 12 in.	2·51	Padegaon-B
Highland acid soil on old alluvium from Govt. Farm at Latekujan (Assam) collected at a depth of 0 — 6 in.	2·47	Latekujan-F
Black cotton soil (neutral, calcareous) from Satara (Bombay) collected at a depth of 0 — 6 in.	2·50	I

### RESULTS AND DISCUSSION

*Relation between the amount of displaced Al, the titratable acidity of the filtrate and the amount of cation adsorbed*

Increasing amounts of  $\text{BaCl}_2$  were added to hydrogen clay sols H and L and estimations were made of (i) the total acidity of the supernatant liquid above the coagula of the sol +  $\text{BaCl}_2$  mixture, (ii) the amount of displaced Al and (iii) the amount of Ba adsorbed. The results are shown in Table I.

The sol and salt mixture was centrifuged after 24 hours from the time of adding the salt to the sol.

TABLE I

*Amounts of Al displaced by  $\text{BaCl}_2$  from sols H and L, the titratable acidity of the filtrate of sol +  $\text{BaCl}_2$  mixtures as also the amounts of Ba adsorbed*

Hydrogen clay	Conc. of $\text{BaCl}_2$	pH of mixture	pH of centrifugate	M.e. per 100 gm. colloid		
				Al* in supernatant liquid	Ba adsorbed	Total acid of supernatant liquid
H	0·01N	3·41	3·57	3·1	11·1	11·4
	0·02N	3·37	3·50	4·9	12·7	11·9
	0·04N	3·38	3·52	8·2	15·2	14·4
	0·09N	3·36	3·35	12·6	18·5	17·0
	1·0N	3·19	3·33	22·0	24·0	22·0
L	0·02N	..	3·27	..	13·0	12·0
	0·04N	..	3·25	10·7	13·8	15·0
	0·09N	..	3·23	12·6	17·7	19·0
	1·0N	..	3·05	17·1	18·8	19·3

\*Fe could not be detected in the supernatant liquid.

At low concentrations of the added salt the total acidity of the supernatant liquid (i.e. exchange acidity) cannot be wholly accounted for by the amount of Al present. With increasing concentration of  $\text{BaCl}_2$  more and more  $\text{Al}^{+++}$  ions are liberated and at a concentration of  $1.0N$   $\text{BaCl}_2$ , the liberated Al, the adsorbed Ba and the exchange acidity have nearly identical values. A fair agreement between the total acidity of the supernatant liquid and the amount of Ba adsorbed is observed. The  $pH$  values of the mixtures containing  $0.02N$ ,  $0.04N$ , and  $0.09N$   $\text{BaCl}_2$  do not differ widely but the amount of Al in the supernatant liquid steadily increases with the concentration of  $\text{BaCl}_2$ . It appears that the free acid developed on the addition of neutral salts does not play a prominent role in the liberation of Al. A direct exchange of both  $\text{Al}^{+++}$  and  $\text{H}^+$  ions for  $\text{Ba}^{++}$  ions offers a more plausible explanation.

*Relation between the pH of the sol and salt mixture and the amount of displaced Al*

Hydrochloric acid is generated in the interaction between the hydrogen clay and the added  $\text{BaCl}_2$ . In order to examine the extent to which Al is dissolved by free HCl, normal HCl was added drop by drop till its  $pH$  became almost equal to that of 'sol and salt' mixture. The amounts of Al in the supernatant liquids above 'sol and HCl' mixtures are given in Table II.

TABLE II

*Amounts of Al displaced by HCl and  $\text{BaCl}_2$  respectively at almost same pH*

Sol	pH of the mixture		M.e. Al in the supernatant liquid per 100 gm. colloid	
	Sol and salt	Sol and acid	Sol and salt	Sol and acid
H . . . . .	3.19	3.07	22.0	1.9
L . . . . .	3.0	3.0	17.1	1.0
Padegaon-B . . . . .	2.54	2.52	40.9	7.5

At the same  $pH$  the amount of Al brought into solution by HCl constitutes a small fraction of that liberated by  $\text{BaCl}_2$ . By far the major portion of the Al liberated by the neutral salt cannot, therefore, be attributed to any dissolution of aluminium oxide by the free acid developed in the salt extract. Paver and Marshall [1934] obtained similar results. They found that at equal strengths (normality) the amount of Al liberated by  $\text{BaCl}_2$  is almost double of that liberated by HCl.

The addition of the salt to the sol lowers its  $pH$ . Experiments were carried out in which the concentration of  $\text{BaCl}_2$  was gradually increased but the  $pH$  was maintained practically constant by using a buffer. Sodium acetate-acetic acid buffer has been used and the results are given in Table III.

TABLE III

*Effect of pH on the liberation of Al by BaCl<sub>2</sub> from sol Padegaon-B*  
(pH of the sol (Padegaon-B) 3.70, colloid content 35.1 gm./l, time of interaction 24 hours)

System	With buffer			Without buffer		
	Conc. of salt	pH	M.e. Al per 100 gm. colloid	Conc. of BaCl <sub>2</sub>	pH	M.e. Al per 100 gm. colloid
25 c.c. sol . . .	0.04N BaCl <sub>2</sub>					
+ 23 c.c. buffer . .	+	3.70	10.0	0.04N	2.61	11.4
+ 2 c.c. N BaCl <sub>2</sub> . .	0.018N Na-Ac.					
25 c.c. sol . . .	0.10N BaCl <sub>2</sub>					
+ 20 c.c. buffer . .	+	3.60	20.0	0.10N	2.57	20.9
+ 5 c.c. N BaCl <sub>2</sub> . .	0.016N Na-Ac.					
25 c.c. sol . . .	1.0N BaCl <sub>2</sub>					
+ 25 c.c. buffer . .	+	3.64	40.9	1.0N	2.50	40.9
+ 3.1 gm. BaCl <sub>2</sub> . .	0.02N Na-Ac.					

Na<sup>+</sup> ions are introduced into the system along with the buffer. Their concentration, however, remains practically constant and variations in the amount of Al liberated should be ascribed to the changing concentration of Ba<sup>++</sup> ions. Besides, the capacity of Na<sup>+</sup> ions to liberate Al has been found [Chatterjee and Paul, 1942] to be very small compared with that of Ba<sup>++</sup> ions. Table III shows that the amount of displaced Al increases steadily with the concentration of BaCl<sub>2</sub>. At any given concentration of BaCl<sub>2</sub>, the amount displaced is independent of the variation of pH observed with and without the buffer. These observations support the postulate of a direct exchange of Al<sup>++</sup> ions.

Na<sup>+</sup> ions introduced along with the buffer may, however, give rise to certain complications. 'Ionic antagonism' is known [Freundlich, 1914; Freundlich and Scholz, 1922; Mukherjee and Ghosh, 1924; also unpublished work of Mitra] to play an important part in reactions in colloidal systems involving more than one type of ions carrying a similar charge. In order to avoid these complications, experiments were carried out in which the pH of the hydrogen clay and BaCl<sub>2</sub> mixtures was adjusted at a practically constant value (3.8) by adding the requisite amounts of Ba(OH)<sub>2</sub>. The results obtained with sol Latekujan-F are given in Table IV.

TABLE IV

*Amounts of Al displaced by BaCl<sub>2</sub> from sol Latekujan-F at a constant pH as also when the pH is not adjusted with Ba (OH)<sub>2</sub>*

Sol + BaCl <sub>2</sub>			Sol + BaCl <sub>2</sub> + Ba(OH) <sub>2</sub>		
Conc. of Ba <sup>++</sup>	pH	M.e. Al displaced per 100 gm. colloid	Conc. of Ba <sup>++</sup>	pH	M.e. Al displaced per 100 gm. colloid
0·0016N . . .	3·70	0·63	0·00162N	3·8	1·0
0·0032N . . .	3·54	2·2	0·0033 N	3·8	1·8
0·008 N . . .	3·40	4·3	0·0082 N	3·8	4·1
0·016 N . . .	3·30	5·1	0·0163 N	3·8	6·2
0·070 N . . .	3·07	15·0	0·0706 N	3·8	15·3

The concentration of Ba<sup>++</sup> ions does not increase materially on the addition of Ba(OH)<sub>2</sub>. The amount of Al liberated at pH 3·8, however, increases with the concentration of the added BaCl<sub>2</sub> and it seems that the pH of the mixture is not of much consequence in determining the amount of liberated Al at a given concentration of Ba<sup>++</sup> ions.

*The effect of removal of the free sesquioxides contained in the hydrogen clay on the quantity of Al displaced by neutral salts*

If Al were liberated as a result of secondary dissolution of Al<sub>2</sub>O<sub>3</sub> contained in the hydrogen clay, a decrease in the amount of Al liberated would be observed on the removal of the free sesquioxides by suitable methods. The free sesquioxides of hydrogen clay Padegaon-B were removed by the methods of Tamm [1922] and Truog [1936]. The amounts of Al liberated by 0·1N BaCl<sub>2</sub> before and after the removal have been compared in Table V.

TABLE V

*Amounts of Al displaced by 0·1 N BaCl<sub>2</sub> from sol Padegaon-B before and after the removal of free sesquioxides*

System	M.e. Al liberated per 100 gm. colloid
Padegaon-B . . . . .	20·9
Padegaon-B (after treatment according to Tamm's method) .	25·4
Padegaon-B (after treatment according to Truog's method) .	36·0

The results show that the amount of Al liberated is not reduced on the removal of the oxides. On the contrary, an increase (calculated per 100 gm. of the residual colloid) is observed. This increase is in agreement with the assumption that the amount of active material per 100 gm. increases on the

removal of free oxides which are really inert instead of being the source of liberated Al. This observation is in agreement with the observed increase in the base exchange capacity of hydrogen clay sols on the removal of the free sesquioxides by the method of Truog *et al.* [unpublished work of Mitra]. When Tamm's method was used the changes in the base exchange capacity were irregular. With some sols an increase was observed, while others showed a decrease. This necessitates a systematic study on the effect of the removal of the free sesquioxides on the displacement of aluminium. Further work on this topic is in progress.

*Effect of time on the amounts of (i) the cation adsorbed, (ii) the exchange acidity and (iii) the Al liberated*

Kappen [1929] observed that the reaction between an acid soil and a neutral salt proceeded so quickly that the pH measured at definite intervals since the beginning of the reaction showed no material variations. This fact has been used by Kappen as an evidence against the secondary dissolution of Al. The idea of an exchange adsorption mooted by Kappen has been contradicted by Page [1926] who is of opinion that 'there is in the liquid phase in contact with the soil absorptive material, at any given degree of unsaturation of the latter, an equilibrium concentration not only of hydrogen ions, but also of aluminium hydroxide, and that both these concentrations increase together.' Paver and Marshall [1934] observed that the Al liberated was almost equivalent to the exchange acidity for shorter periods but showed a distinct fall later. This decrease in the Al liberated was accompanied by a fall in the pH. They consider that in the later stages a small amount of aluminium hydroxide was being adsorbed and a corresponding amount of acid liberated. In the light of their results the effect of time on the amounts of (i) Ba adsorbed, (ii) the exchange acidity and (iii) liberated Al has been studied with hydrogen clay sols H and I using 0.09N BaCl<sub>2</sub>. The results are given in Table VI.

TABLE VI

*Variations with time in the amounts of (i) the cation adsorbed, (ii) the exchange acidity and (iii) the liberated Al in the case of sols H and I using 0.09 N BaCl<sub>2</sub>*

Sol	Time allowed	pH of the centri-fugate	M.e. Al in the centri-fugate per 100 gm. colloid	M.e. Ba adsorbed per 100 gm. colloid	M.e. ex-change acidity per 100 gm. colloid
H (SiO <sub>2</sub> /R <sub>2</sub> O <sub>3</sub> = 2.1)	5 mins. . .	3.28	12.5	18.1	18.0
	30 „ . .	3.06	15.6	20.6	23.0
	6 hours . .	3.14	13.6	19.9	19.1
	24 „ . .	3.33	12.6	18.6	17.0
	48 „ . .	3.30	12.2	18.5	17.2
I (SiO <sub>2</sub> /R <sub>2</sub> O <sub>3</sub> = 2.5)	5 mins. . .	2.75	26.4	35.1	34.0
	30 „ . .	2.74	27.5	34.9	32.0
	6 hours . .	2.70	26.9	..	34.0
	24 „ . .	2.70	26.1	32.0	34.0

With sol H the Al liberated, adsorbed Ba and the acid displaced all increase at first. A decrease is then observed and finally they become constant. These results are in agreement with those obtained by Paver and Marshall [1934] who, however, measured only the variations of the amount of Al displaced and the pH of the salt extract. The amount of the cation adsorbed and the acidity developed were not estimated. Their observations do not show the initial increase in the amount of Al displaced. The data cited in Table VI show in addition that the decrease in the amount of Al displaced is not accompanied by a fall in pH nor with any increase in exchange acidity. And it appears that the assumption made by Paver and Marshall [1934] of a subsequent adsorption of a small quantity of aluminium hydroxide resulting in the liberation of a corresponding amount of acid is not adequate. A decrease in the amounts of (i) displaced Al, (ii) adsorbed Ba and (iii) the acid displaced in the later stages suggests that after some time some re-adsorption of  $Al^{+++}$  ions takes place accompanied by a 'desorption' of the adsorbed  $Ba^{++}$  ions. With sol I no material variations in (i), (ii) and (iii) with time were noticed. This observation is in agreement with that of Kappen [1929]. It appears that the discrepancy in the results obtained by different workers probably arises from the use of different types of soils. The electrochemical properties of sols H and I have also been found to differ in several important points [Mitra, 1940].

#### SUMMARY

1. The acid liberated on the addition of a neutral salt to a hydrogen clay sol cannot be wholly accounted for by the amount of Al present in the salt extract when low concentrations of the salt are used. The amount of Al displaced increases with the concentration of the salt.

2. The titratable acid of the  $BaCl_2$  extract and the amount of Ba adsorbed by the hydrogen clay are in fair agreement.

3. At the same pH, the amount of Al brought into solution by HCl constitutes a small fraction of that liberated by  $BaCl_2$ . Practically the same amount of Al is liberated when both the pH of the sol decreases as the result of the addition of the salt as also when the pH is kept constant by the use of a suitable buffer, or by adding the requisite amount of the corresponding base.

4. The amount of Al displaced does not decrease on the removal of free sesquioxides contained in the hydrogen clay but increases.

5. The time that elapses after addition of the salt to the sol has some effect on the amount of Al found in the salt extract. The time effect appears to be influenced by the type of soil from which hydrogen clay is obtained.

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## II. THE ROLE OF ALUMINIUM IN RELATION TO THE FREE AND TOTAL ACIDS OF HYDROGEN CLAYS\*

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(With six text-figures)

**I**NVESTIGATIONS with hydrogen clay sols in this laboratory [Mitra, 1936 ; Mukherjee *et al.*, 1937 ; Mitra *et al.*, 1940 ; Mitra, 1940] show that : (i) the free acidity of a hydrogen clay sol, calculated from the pH value usually constitutes a small fraction (3-10 per cent) of its total neutralizable acid, determined from the inflexion point in the titration curve with a base, (ii) the amounts of acid which interact with different bases are in the order  $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{KOH} > \text{NaOH}$ , (iii) the total neutralizable acid of a hydrogen clay sol is greatly increased on the addition of neutral salts, (iv) the total acidity of the supernatant liquid above the coagula of the 'sol and salt' mixture is considerably less than that of the suspension as a whole or that of the pure sol, and (v) the amount of acid displaced into intermicellary liquid depends on the electrical adsorbability of cations which is determined [Mukherjee, 1921, 1922] by their mobility and valency.

In the previous part [Mukherjee and Chatterjee, 1942] it has been shown that both exchangeable  $\text{H}^+$  and  $\text{Al}^{+++}$  ions are present on the surface of the colloidal particles. The present investigation has been undertaken with a view to obtaining definite information regarding the role of these  $\text{Al}^{+++}$  ions on the free and total acids of a hydrogen clay sol.

The methods of preparation and purification of the sols and the experimental procedure have been described by Mukherjee and Chatterjee [1942].

### RESULTS AND DISCUSSION

*Features of the titration curves of the sol, of the 'sol and salt' mixtures and their clear supernatant liquids*

The potentiometric titration curve (Fig. 1) of the sol Latekujan-F with NaOH reveals a weak dibasic acid character. The  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  curves (Figs. 2 and 3) on the other hand, resemble that of a weak monobasic acid. The form of the curve changes when a salt has been added and with

\*The results have been taken from the published Annual Reports for 1940-41 on the working of a 'Scheme of Research into the Properties of Colloid Soil Constituents' financed by the Imperial Council of Agricultural Research, India and directed by Prof. J. N. Mukherjee.

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increasing concentrations of the salt the form becomes progressively characteristic of a strong acid. The flocculation caused by salts leaves, after some time, a clear supernatant liquid whose titration curves (Figs. 4, 5 and 6) have forms widely differing from those of the sol and salt mixtures. The initial portion becomes steep and merges into a region of noticeable buffering which becomes prominent with increasing concentration of the salt. The buffering occurs between  $pH$  3.75 and 5.0 and merges in its turn into a second steep portion showing an inflexion point characteristic of the neutralization point of an acid or a base. These features have been observed with solutions of aluminium salts [Britton, 1927].

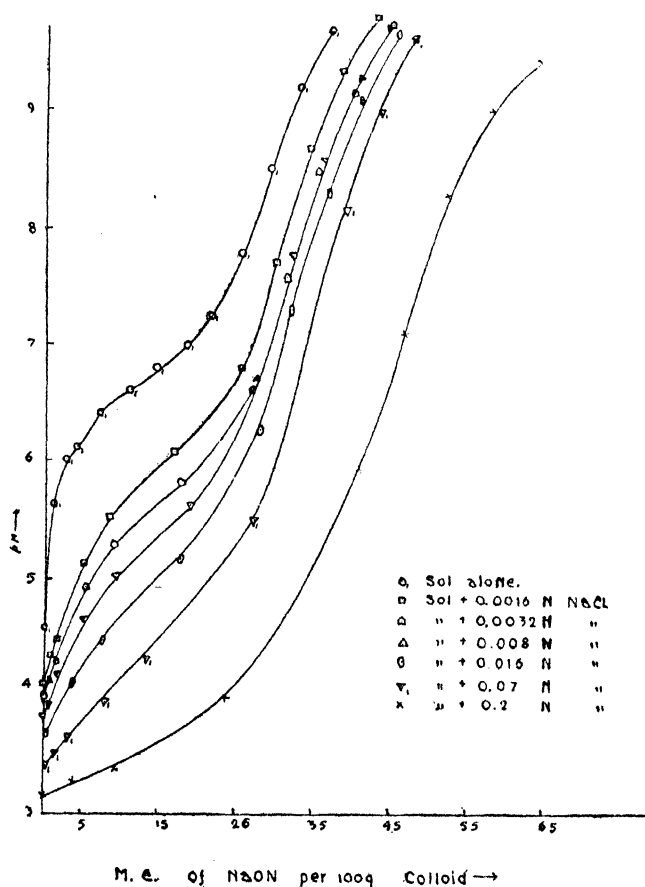


FIG. 1. Potentiometric titration curves of the sol Latekujan-F with NaOH

*Relation between the total acidity of the hydrogen clay and salt mixture, the total acidity of the supernatant liquid and the amount of Al displaced*

Table I records the total reacting acid of the sol Latekujan-F, of its mixtures and with salts having six different concentrations of NaCl and BaCl<sub>2</sub>, and of the supernatant liquids and the quantities of displaced Al in the supernatant liquids. Similar data for sol H and its mixtures with five different concentrations of BaCl<sub>2</sub>, are also given in the same table.

TABLE I.

*Total acidities of sols Latekujan-F and H and salt mixtures and those of their supernatant liquid and the amounts of displaced Al*

Sol	Concentration of salt (N)	M.e. total acid* per 100 gm.				M.e. Al displaced per 100 gm.	Average	a-b	a-c	c-d
		Sol + salt	Sol	Super-natant liquid	Average					
		a	b**	c	c	d	d			
Latekujan-F	0.0016 NaCl	30.0	28.0	0.18 0.22	0.20	Nil		2.0	29.8	+0.2
	0.0032 "	31.5	"	0.32 0.28	0.30	0.34 0.30	0.32	3.5	31.2	-0.02
	0.008 "	32.0	"	0.62 0.58 0.60	0.60	0.62 0.55	0.58	4.0	31.4	+0.02
	0.016 "	32.5	"	1.8 1.9	1.85	1.20 1.25	1.22	4.5	30.6	+0.63
	0.070 "	34.5	"	4.1		2.68 2.60	2.64	6.5	30.4	+1.47
	0.20 "	44.0	"	10.0		11.0		16.0	34.0	-1.0
Latekujan-F	0.0016 BaCl <sub>2</sub>	31.0	30	1.4 1.4	1.4	0.66 0.61	0.63	1.0	29.6	+0.77
	0.0032 "	32.0	"	3.2		2.1 2.2	2.15	2.0	28.8	+1.05
	0.008 "	38.0	"	4.8		4.3		3.0	28.2	+0.5
	0.016 "	34.5	"	7.0		5.1		4.5	27.5	+1.9
	0.070 "	42.0	"	16.0		15.0		12.0	26.0	+1.0
	0.20 "	50.0	"	22.4		19.0		20.0	27.6	+3.4
H	0.01 BaCl <sub>2</sub>	33.0	28.5	11.0		3.0 3.2	3.1	4.5	21.6	+8.3
	0.02 "	35.0	"	12.0		4.9		6.5	23.0	+7.1
	0.04 "	37.6	"	14.4		8.2		9.0	23.1	+6.2
	0.09 "	43.0	"	17.0		12.6		14.5	26.0	+4.4
	1.0 "	44.0	"	22.0		22.0		15.5	22.0	0

\*Calculated at the inflexion point in the titration curve with corresponding bases.

\*\*Calculated at the second inflexion point in the titration curve with NaOH.

The total acidities decrease in the following order : ' sol and salt ' mixture (i) > sol (ii) > supernatant liquid of the sol and salt mixture (iii).

Excepting the lower concentrations of NaCl\* in the case of sol Latekujan-F and the highest concentration of BaCl<sub>2</sub> in the case of sol H, the total acidities of (iii) are definitely greater than their Al contents. This excess is given by the difference between the values under c and d in Table I where it

\*At the lower concentrations of NaCl the difference between the two quantities is within the limits of experimental error. The same holds for 0.2N NaCl and the two quantities are exactly equal at a concentration of 1.0N BaCl<sub>2</sub> in the case of sol H.

is shown under  $c-d$ . It should be ascribed to hydrogen ions displaced from the double layer into the intermicellary liquid. The amount of displaced hydrogen ions is not so prominent with Latekujan-F as it is with H.

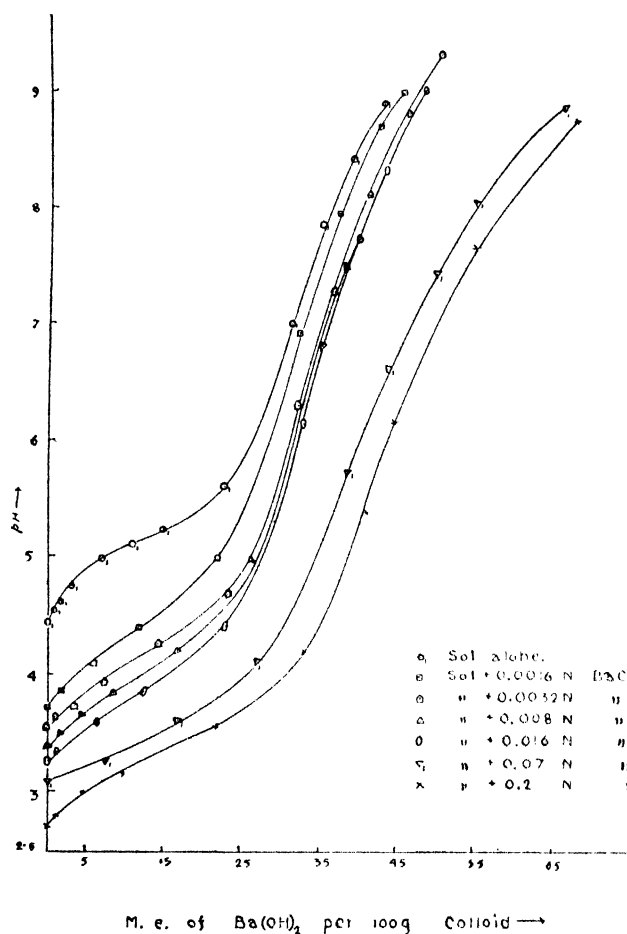


FIG. 2. Potentiometric titration curves with  $\text{Ba}(\text{OH})_2$  of the sol Latekujan-F and of its salt mixtures

The variations in  $c-d$  with concentration of the added salt are rather irregular with sol Latekujan-F. The errors involved in the estimation of  $c$  and  $d$  which are small quantities, especially at the lower concentrations of the added salts, are magnified in the difference between them. The quantity  $c-d$  for Latekujan-F has, on the whole, a tendency to rise with increasing salt concentrations but it shows a constant decrease in the case of sol H. Unpublished work of Mitra from this laboratory also shows that the two sols have widely different electrochemical properties. This contrast is probably associated with the difference between the two soils from which the respective hydrogen clays have been prepared ; sol H from neutral calcareous soil from Government Seed Farm, Kalyanpore (United Provinces) and sol Latekujan-F from

highland acid soil on old alluvium from Government Farm at Latekujan (Assam).

It is evident from the preceding and also from the work of Mukherjee and Chatterjee [1942] that both  $H^+$  and  $Al^{+++}$  ions are present in the double layer associated with the colloidal particles and both are displaced into the intermicellary liquid on the addition of neutral salts, but at higher concentration of the salt  $Al^{+++}$  ions form the major constituent.

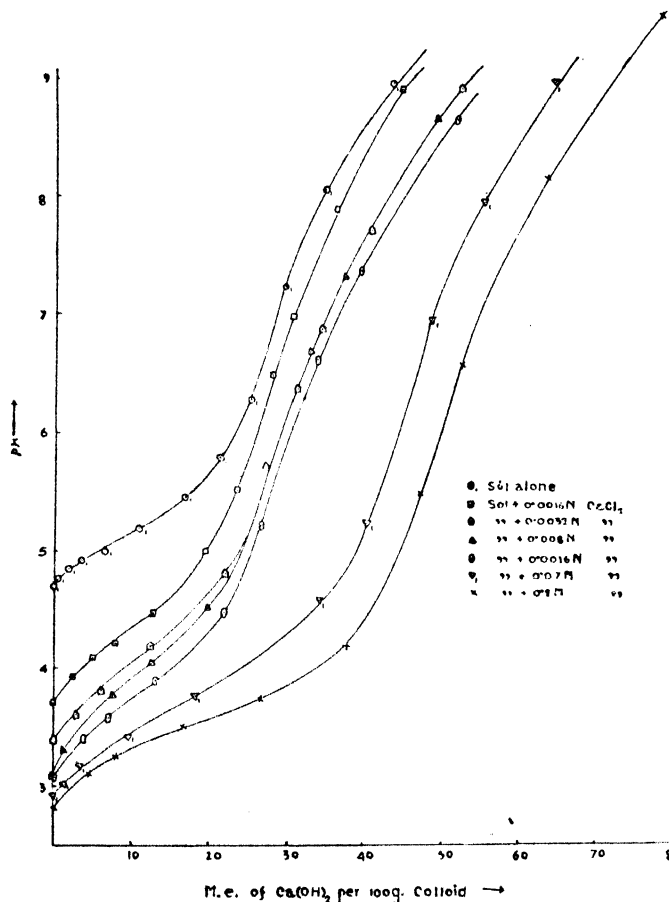


FIG. 3. Potentiometric titration curves with  $Ca(OH)_2$  of the sol Latekujan-F and of its salt mixtures

The increase in the total acidity of a hydrogen clay sol on the addition of neutral salts indicates that more ions,  $Al^{+++}$  and/or  $H^+$  ions, in addition to those already present in the interface are brought into a reactive condition. In order to ascertain to what extent these ions are displaced in the intermicellary liquid or remain associated with the colloidal particles the total acidities of the sol, of the 'sol and salt' mixtures and their supernatant liquids have been compared (Table I). The total reacting acids of the 'sol and salt' mixtures and of the supernatant liquid both increase progressively with the gradual

addition of a salt. The total acidity of the supernatant liquid, even for normal salt solutions is, however, less than that of the sol itself. Obviously all the active ions originally present on the surface are not displaced by the salt under these conditions. The larger total acidity of the 'sol and salt' mixtures compared to that of the sol itself definitely shows, however, that additional ions having a higher affinity for the surface have been rendered active but remain associated with the colloidal particles. When NaCl is the salt used, the difference  $a-b$ , (Table I), which gives a measure of the additional amounts of ions rendered active, is greater than  $c$ , the total acidity of the supernatant liquid. In agreement with the weak displacing power of  $\text{Na}^+$  ions, the amount of ions rendered reactive is thus not completely displaced in the intermicellary liquid. Consistent with the strong power of displacement of  $\text{Ba}^{++}$  ions  $a-b$  is less than  $c$ .

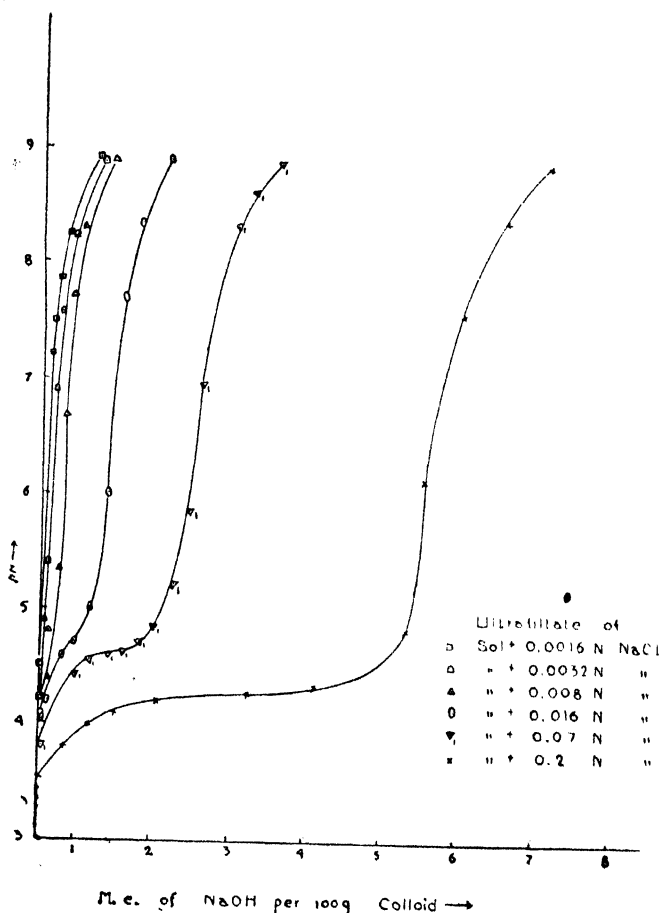


FIG. 4. Potentiometric titration curves with  $\text{Na}(\text{OH})$  of the ultrafiltrates of the soil Latekujan-F and NaCl mixtures

In order to ascertain the amount of these ions remaining associated with the surface in presence of salts the difference  $a-c$ , between the total acidity of the 'sol and salt' mixtures and that of the corresponding supernatant

liquid has been given in Table I. While  $\alpha-c$  is not constant, it does not differ greatly on the addition of salts or from the total acid of the sol itself. The quantity  $\alpha-c$  appears to depend on the displacing power of the cation of the salt and an equilibrium between the ions in the intermicellary liquid and in the double layer is indicated.

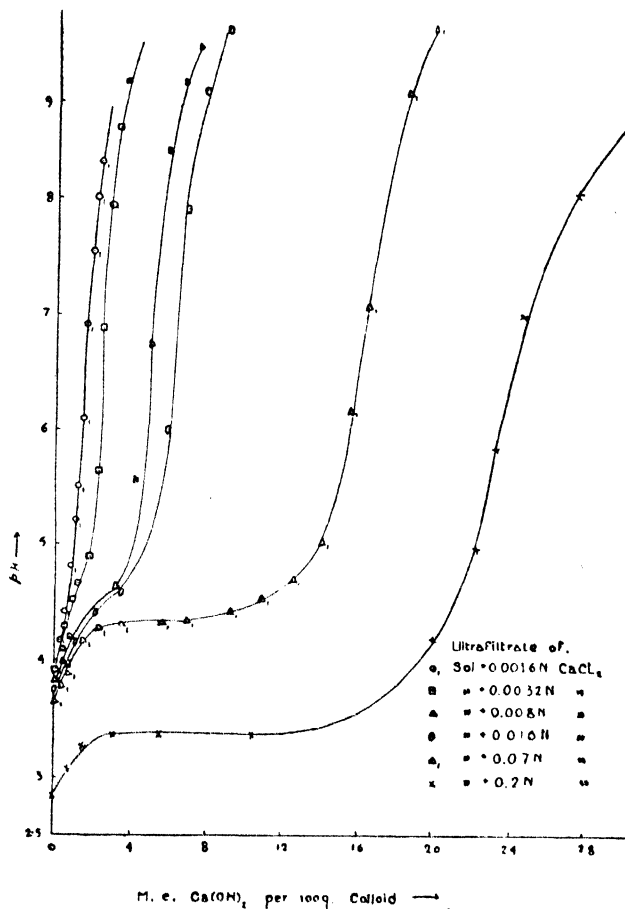


FIG. 5. Potentiometric titration curves with  $\text{Ca(OH)}_2$  of the ultrafiltrates of the sol Latekujan-F and  $\text{CaCl}_2$  mixtures

#### SUMMARY

Both  $\text{H}^+$  and  $\text{Al}^{+++}$  ions are present on the surface of the colloidal particles of hydrogen clay,  $\text{H}^+$  ions constituting a small fraction of the total. Of the total amount of these ions a portion is displaced into the intermicellary liquid on the addition of a neutral salt, while another portion remains associated with the colloidal particles. With increasing salt concentrations more and more ions ( $\text{H}^+$  and  $\text{Al}^{+++}$ ) are displaced into the supernatant liquid but fresh ions are brought into a reactive condition. The amount of ions remaining associated with the colloidal particles depends on the displacing power of the cation. A large reservoir of these ions on the surface is indicated.

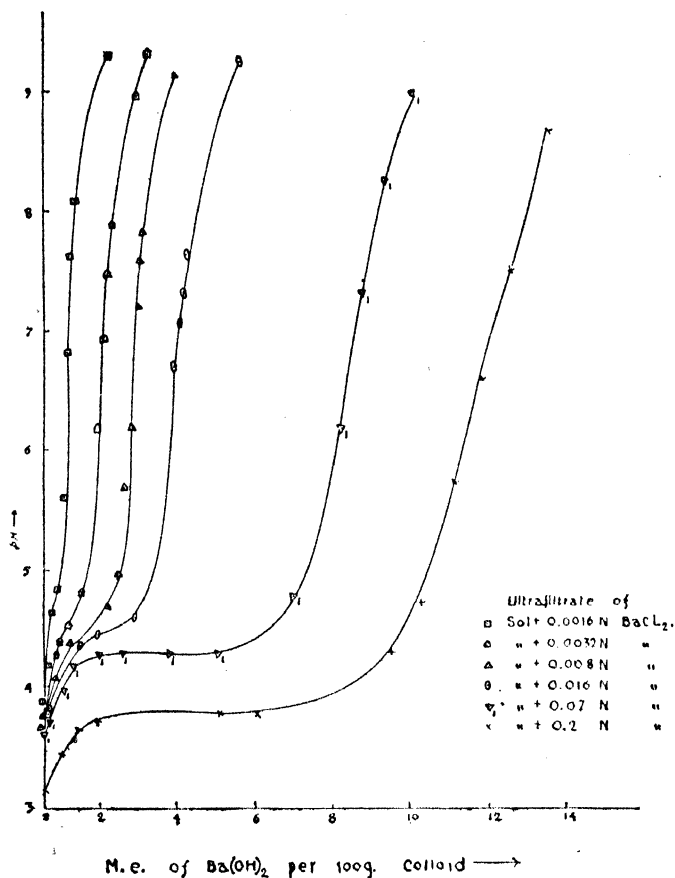


FIG. 6. Potentiometric titration curves with  $\text{Ba}(\text{OH})_2$  of the ultrafiltrates of sol Latekujan-F and  $\text{BaCl}_2$  mixtures

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# SOILS OF THE DECCAN CANALS

## II. STUDIES IN AVAILABILITY OF NITROGEN IN SOIL WITH APPLICATION OF FARMYARD MANURE UNDER DIFFERENT CONDITIONS OF MOISTURE AND CARBON/NITROGEN RATIOS

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(With two text-figures)

THE application of farmyard manure in crop production is an essential item in Indian agriculture and is no exception under sugarcane growing in the canal zones of the Bombay-Deccan, where large quantities of the manure are frequently used for this crop. Yet, in spite of the long-established practice of the use of farmyard manure in India, comparatively little scientific information is available regarding its efficacy in supplying available nitrogen to crops, or its ultimate effect in modifying soil properties, particularly the biological conditions.

In western countries the considerable amount of literature available on the subject has been reviewed by Jensen [1931]. Among recent work in India mention may be made of the investigation of Mukerji and Vishnoi [1936] on the rice soils of Raipur (Central Provinces). They have shown that the rate of decomposition of farmyard manure under submerged conditions approximates to that under aerobic condition and is higher in a medium clay than in a sandy loam soil. Mirchandani [1932] has stressed the importance of the C/N ratios in influencing the mineralization of farmyard manures in soils. Bal [1935], working on the rate of decomposition of added organic matter on the heavy black cotton soils of the Central Provinces, finds that the biological activities are at their best when the moisture content is about half the maximum water-holding capacity. Recently, Vishwanath [1937] has found heavy losses of nitrogen occurring under field conditions at Coimbatore during the nitrification of added ammonium sulphate, green manure and cattle manure, the loss being greatest with ammonium sulphate. In spite of the heavy losses there was no movement of nitrogen into the deeper layers and there was no moisture saturation leading to denitrification. On the contrary, it has been observed in uncropped irrigated plots at the Sugarcane Research Station, Padegaon (unpublished data), that, with a heavy dressing of farmyard manure (60,000 lb. per acre), there was actually fixation of nitrogen to the extent of 74 per cent over the original within six months. But there was very little nitrification, the nitrate levels of the manured plots

\* This scheme is partly subsidised by the Imperial Council of Agricultural Research.

being not appreciably higher than those of the control during this period of experimentation. Similarly, from replicated experiments with cane conducted at the same station for three years, there is reason to believe that the contribution of farmyard manure to nitrogen nutrition of the crop is negligible, whereas its beneficial effect in creating a desirable soil-tilth is quite manifest in the case of a shallow-rooted cane variety [Rege, 1941].

The most important aspect of this question, however, is that, in sugar-cane farming, where it has been the practice to add heavy dressings of farmyard manure, there would not only be an enormous waste of the manure if indiscriminately used, but also, according to indications obtained in the course of a fertility survey of cane soils in the Deccan, the ultimate effect of such a practice would lead to soil deterioration by the widening of the C/N ratio under such conditions. The results of the above-mentioned survey showed that cane soils showing signs of deterioration, i.e. where more and more nitrogen is required every year to produce the same yields, have in the majority of cases higher C/N ratios than the normal fertile soils. It was, therefore, felt that the solution of this problem would have an important bearing on the economics of cane growing and on the equally important question of the maintenance of soil fertility.

Accordingly, pot-culture experiments were carried out during the years 1935-37 to investigate fully the availability of the manure in different soils and its ultimate effect on their fertility status. In the present paper this question will be dealt with from the points of view of moisture conditions and C/N ratios in one of the soil types which occurs at the farm.

## EXPERIMENTAL

### *Soils and manures used*

The soils used in these experiments belong to the group of typical black cotton soils which overlie the Deccan Trap—a volcanic formation of basaltic rocks. Recently they have been classified according to the modern genetic system [Basu and Sirur, 1938] and the present work deals with the decomposition of manures in one of the important soil types—called the 'B' type, a brief description of which is given below :—

### *The profile*

Horizons	Description
I	Uniform dark grey with a brown shade, interspersed with roots, clay loam :— (a) Large clods, 2-3 in. in diameter. (b) Smaller clods $\frac{1}{2}$ -1 in. in diameter, more friable than above
II	Mottled horizon, brown intermingled with greyish black, brown predominating in lower layers, silt loam.
III	Reddish brown colour, with white concretions of lime and silicate material—more compact than above—clay.
Below	Hard murrum (decomposed trap).

The depth of soil above the murrum varies from about 3 to 12 ft.

### *Soil characteristics*

In the present work only the first foot of soil has been used for the experiment. Some important characteristics of this depth are given in Table I.

TABLE I  
*General characteristics of the soil*

## (A) Bulk chemical analysis

Residue insoluble in HCl (per cent)	Soluble SiO <sub>2</sub> (per cent)	Fe <sub>2</sub> O <sub>3</sub> (per cent)	Al <sub>2</sub> O <sub>3</sub> (per cent)	CaO (per cent)	MgO (per cent)	K <sub>2</sub> O (per cent)	Na <sub>2</sub> O (per cent)	P <sub>2</sub> O <sub>5</sub> (per cent)
50.29	14.74	10.40	14.55	7.14	1.48	0.110	0.957	0.075

## (B) Other property

Mechanical analysis		Exchangeable bases m. e. per cent				pH value		Calcium carbonate per cent
Clay per cent	Silt per cent	Ca	Mg	K	Na	in water	in N KCl	
61.75	14.75	44.25	10.87	3.96	4.10	8.42	7.51	9.01

The general nature of the locally available farmyard manures will be seen from Table II where analytical data for seven typical manures and one sample of compost (i.e. No. 7) prepared at the Padegaon Farm are given. The farm-yard manures are usually prepared from the waste materials of *jowari* (*Andropogon sorghum*) straw left after feeding the cattle, together with their urine and dung, while compost has been prepared out of sugarcane trash.

TABLE II  
*General analyses of farmyard manures and compost*

Sample No.	Age of manure in months	Loss on ignition	Carbon	Nitrogen	Humus	Per cent humified matter	C/N ratio	pH in water
		Per cent on air-dry basis						
1	2	3	4	5	6	7	8	9
1	6	29.2	12.15	0.847	6.15	29.4	14.3	7.37
2	12	21.7	8.61	0.739	7.25	48.9	11.7	7.45
3	12	19.7	5.85	0.582	3.43	34.0	10.1	8.12
4	10	26.1	9.98	0.946	5.72	33.2	10.6	7.45
5	9	21.3	6.06	0.606	4.91	47.0	10.0	7.95
6	8	27.5	7.04	0.727	8.63	71.1	9.7	7.12
7	8	35.1	7.53	0.811	6.98	53.8	9.3	7.20
8	24	11.2	5.17	0.483	4.77	53.5	10.7	7.20

It will be noticed that the C/N ratios are usually round about 10—unless the sample is taken too raw as in No. 1—and the nitrogen content varies from 0.48 to 0.95 per cent. The per cent humified matter varies from 29.4 to 71.1 and does not seem to bear any well-defined relation with the age of the manure.

*Technique followed*

The decomposition studies described in this paper were conducted under conditions corresponding to those under alternate wetting and drying of the soil as occurs under sugarcane cultivation in the Deccan. Fifteen kilograms of air-dried soil were used in this experiment after thorough mixing of the required quantities of manures and distilled water. The treated soils were placed in glazed earthenware pots (1 ft. diameter  $\times$  1 ft. height) and packed so as to occupy a constant volume in each pot. The pots were then left exposed to the atmospheric conditions in a room. The pots were weighed at regular intervals to find out the loss by evaporation. Representative soil samples for the various determinations were taken after thorough mixing of the soil. The required quantity of water was added to the soils with proper stirring and the packing adjusted uniformly in all the pots. Care was taken to keep the time of sampling, water addition and plating for bacterial work constant throughout the experiment. Ammoniacal nitrogen, nitrate nitrogen, bacterial number and studies in respiration were done on the fresh soil, the results being calculated on oven-dry soil by keeping a separate sample for moisture determination. Carbon and nitrogen were determined on the air-dried samples and figures are reported on oven-dry basis.

The following analytical methods were employed :—

Ammoniacal nitrogen was determined by extracting the soil by 2*N* KCl at *pH* 1.0 as recommended by C. Olsen [Wright, 1934].

Nitrate nitrogen was determined by the phenol-disulphonic acid method recommended by A. O. A. C. [Methods of Analysis, A. O. A. C., 1930].

Total nitrogen was determined by the routine Kjeldahl method using the modification of Bal [1925].

Carbon was determined by the wet combustion method [Leather, 1907]. It was found, however, that destruction of carbonates was not complete within the period of  $\frac{1}{2}$  hour using water bath. After a number of trials with the black cotton soils of the tract, which are usually highly clayey and calcareous, a prolonged period of heating for two hours was found necessary, however, with the precaution of having a Liebig's condenser attached to the flask throughout the heating in order to avoid excessive concentration of the acid mixture. Also, later, during the heating of the soil with potassium dichromate, an oil bath at a temperature of 130°C. was resorted to, the aspiration being continued for six instead of five hours.

Humus was determined by Sigmond's method, by extraction with *N*/10 sodium carbonate [Sigmond, 1927].

Numbers of bacteria were determined by the plate method, using the agar medium of Thornton [1922] containing  $K_2HPO_4$  1 gm.,  $MgSO_4 \cdot 7H_2O$  0.2 gm.,  $CaCl_2$  0.1 gm., NaCl 0.1 gm.,  $KNO_3$  0.5 gm.,  $FeCl_3$  0.002 gm., asparagine 0.5 gm., mannitol 1 gm., agar 20 gm., water to 1000 c.c. The quantity of agar had to be increased to 20 gm. from the recommended 15 gm. as otherwise the medium was not solidifying under the climatic condition here; *pH* was adjusted in each case to 7.4 as recommended.

Ten gm. of the fresh soil sample were shaken up for four minutes with 250 c.c. of a sterile saline of 0.5 per cent NaCl and 0.05 per cent  $MgSO_4$  (suspension *a*). Subsequent dilutions corresponding to 1/2500 (dilution *b*) and

1/250,000 (dilution *c*) were prepared from suspension (*a*) and (*b*), respectively, by adding requisite quantities of sterile saline. Throughout the experiment, dilutions (*b*) and (*c*) were used for plating, taking 1 c.c. each of the above suspensions in sterile petri dishes and adding to them 10 c.c. lots of sterile medium. The petri dishes were incubated for 5 days (which was found to be the optimum period) at a temperature of 35°C. Five replicates were kept in each case, the average figure being taken for calculation of the bacterial number. The standard error was found to be within 20 per cent in the majority of cases.

*Evolution of CO<sub>2</sub>.*—From the periodical soil samples collected from the pots, 250 gm. were taken in conical flasks and the CO<sub>2</sub>-evolution measured after every 24 hours by absorption in standard baryta solution in Pettenkoffer's tubes by aspiration of a steady stream of CO<sub>2</sub>-free air through the apparatus. The CO<sub>2</sub>-evolution was followed up for a period of nine days during which it came down to a constant and negligible level. The CO<sub>2</sub>-evolution during this nine-day period was then divided by 9 to get the daily average, and these figures were entered against the day of sampling for comparison.

#### DATA AND DISCUSSION

##### *First series of experiments*

In this experiment the decomposition in soil of a sample of farmyard manure (No. 2 of Table II) was studied with the moisture contents of soil at field saturation and at half saturation, the moisture being made up at weekly intervals to 44 per cent and 22 per cent respectively. These moisture levels are found to represent, more or less, the average conditions of moisture obtaining under periodical heavy irrigation usually given to cane, and under the normal rainfall in the tract, respectively. The soil used in this experiment was that of a normal fertile soil obtained from an experimental block on the Padegaon Farm and had a carbon/nitrogen ratio of 14.9 at the time of the experiment.

The treatments were as follows :—

- I. Control soil—no manure.
- \*II. Addition of farmyard manure corresponding to 0.33 per cent of the soil used.
- \*III. Addition of farmyard manure corresponding to 1 per cent of the soil used.

Periodical determinations of bacterial numbers, ammonia and nitrate were conducted every week till 42 days, whereas carbon and total nitrogen were determined once at the start and again after 200 days in order to allow sufficient time for soil changes to take place.

##### *Nitrogen changes and bacterial numbers*

The ammoniacal and nitrate-nitrogen figures for different periods are given in Tables III and IV.

\* These applications of manure work up to 10,000 lb. and 30,000 lb. per acre respectively under field conditions.

TABLE III

*Ammoniacal nitrogen in mg. per 100 gm. of air-dry soil under field and half moisture saturations in control and farmyard manure treated pots*

(Normal fertile soil)

Treatment*		Number of days from commencement						
		0	7	14	21	28	35	42
I. No manure	F.	1.22	1.55	0.60	0.60	2.69	3.56	1.20
	H.	1.22	0.53	0.71	0.73	0.57	0.63	0.52
II. Farmyard manure (0.33 per cent)	F.	1.22	1.54	0.81	0.76	2.84	1.20	1.08
	H.	1.22	1.03	0.71	0.60	0.43	0.68	0.37
III. Farmyard manure (1 per cent)	F.	1.22	1.55	1.25	0.76	3.00	1.17	1.35
	H.	1.22	1.06	0.76	1.02	0.80	0.57	0.41

\*F=Field moisture saturation ; H=Half moisture saturation

Generally speaking, there is more accumulation of ammoniacal nitrogen at field-moisture saturation than at half saturation both in control and manured soils. The levels of ammonia are not much affected by the addition of farmyard manure excepting on one occasion (i.e. 35th day) when considerable lowering in ammonia is observed at field-moisture saturation.

TABLE IV

*Nitrate nitrogen in mg. per 100 gm. of air-dry soil under field and half moisture saturations in control and farmyard manure treated pots*

(Normal fertile soil)

Treatment		Number of days from commencement						
		0	7	14	21	28	35	42
I. No manure	F.	0.16	0.15	1.20	0.94	1.06	0.61	0.86
	H.	0.16	0.10	0.08	0.07	0.10	0.14	0.27
II. Farmyard manure (0.33 per cent)	F.	0.16	0.23	0.16	0.21	0.63	0.92	0.82
	H.	0.16	0.26	0.31	0.22	0.30	0.36	1.93
III. Farmyard manure (1 per cent)	F.	0.16	0.17	0.18	0.23	0.20	1.76	0.96
	H.	0.16	0.51	0.60	0.32	0.35	0.32	2.52

The nitrate contents of soils are also maintained at a higher level at field-moisture saturation than at half saturation but, while farmyard manuring generally lowers the nitrate at the former moisture level, it raises the values, especially on the 42nd day, in both doses of farmyard manure at the latter moisture level.

The relationship between the bacterial number and mineral nitrogen (i.e. ammonia plus nitrate) is given in Fig. 1.

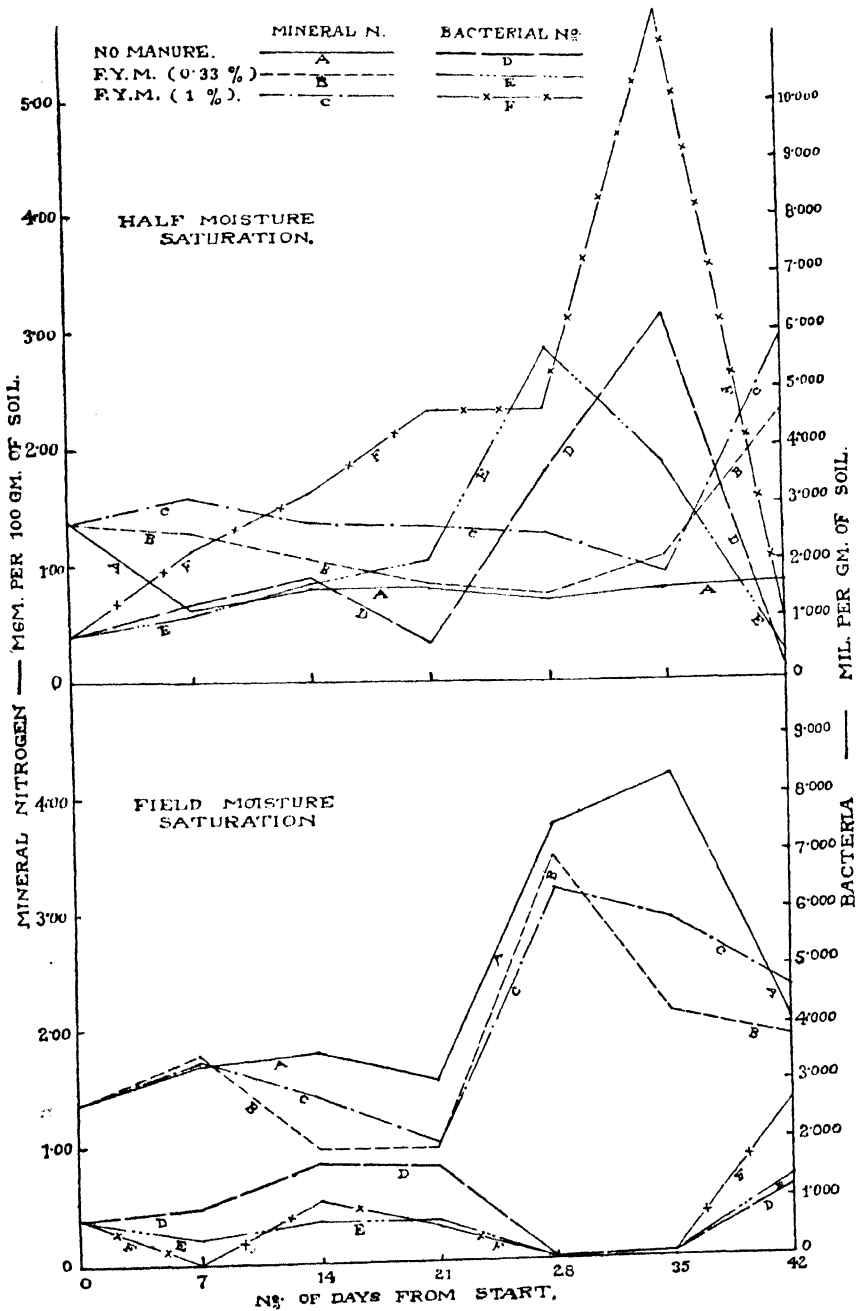


FIG. 1. Periodic fluctuations in mineral nitrogen and bacterial numbers—normal fertile soil

Although mineral nitrogen shows generally a higher level at field saturation than at half-saturation moisture in all the treatments, the effect of addition of manure is quite different at two moisture levels. Thus while farmyard manuring has helped to raise the mineral nitrogen status of soil at half saturation it has affected it adversely at field saturation. This fact is of considerable importance in the economy of sugarcane farming on the Deccan Canals where addition of large amounts of bulky manures is usually practised by the cane cultivators, resulting in their great wastes. This non-availability of nitrogen in farmyard manures on 'B' type\* of soil has been actually supported by experiments conducted at the Padegaon Farm [Rege, 1941].

Referring to Fig. 1, it will be observed that the bacterial activity as measured by plate counts indicates a general lowering in the activity by addition of manure at field-moisture saturation, whereas increased activities are shown by this treatment at half-moisture saturation. This clearly reflects the unfavourable conditions created for bacterial growth by manuring at field moisture. Similar depressing effects of manures on bacterial numbers were also found by Mukerji and Vishnoi [1936] while working on the heavier soils of Raipur, Central Provinces. They have attributed it to the formation of some toxic product which proves harmful to the bacteria capable of growing on Thornton's agar.

One interesting thing to be observed in this connection is the fact that in spite of lower bacterial numbers shown at field moisture the mineralization of nitrogen is quite high when compared with figures at the half-saturation moisture. Apparently the more useful types of organisms which are responsible for mineralization of nitrogen must be present in larger numbers (or in more reactive form) at field moisture but which are not reflected in mere plate counts.

Now, coming to the relationship between the fluctuations in the mineral nitrogen contents at different periods and bacterial numbers, two things must be borne in mind, viz. (a) the mineral nitrogen present at any moment in the soil is the balance between that which is produced by micro-organisms *minus* the amount taken up by microbial cells *plus* the nitrogen lost by denitrification and loss in gaseous forms; (b) the bacterial counts by Thornton's plate method do not include all the micro-organisms, especially the cellulosedecomposing bacteria, and thus any relationship observed between mineral nitrogen and bacterial number must be of a qualitative nature. With these comments we find a fair amount of negative correlations between mineral nitrogen and bacterial numbers. At field-moisture saturation the bacterial numbers generally rise till 21st day after which there is a fall on the 28th and 35th day and a rise again. The mineral nitrogen also shoots up on the 28th and 35th day when the bacterial numbers are very low and falls again when the numbers rise. At half-moisture saturation the bacterial numbers rise up to 28th or 35th day and then attain very low values on the 42nd day. The mineral nitrogen which steadily falls with rise in bacterial number, rises high only when the number goes down very low. This sort of inverse relationship between bacterial number and mineral nitrogen has also been observed by

\* The availability of nitrogen in farmyard manure in other soil types will be discussed in a subsequent paper.

Jensen [1931] at Rothamstead, who attributed the lowering of mineral nitrogen to absorption by the bacterial cells and its release on the death of the microbial bodies resulting in higher production of mineral nitrogen in the soil. It would be thus evident that in the different treatments, the greater the bacterial number prior to its attaining the lowest values, the greater will be the release of mineral nitrogen at the cessation of the bacterial activity. The contrary behaviour of farmyard manuring on the production of mineral nitrogen (at the cessation of bacterial activity) in the two moisture levels are thus clear.

#### *Final changes in soil under different treatments*

The changes in carbon, nitrogen and C/N ratios in soils were determined after 200 days in different treatments and are shown in Table V. The figures in brackets indicate the calculated values taking the original values as 100.

TABLE V

*Final changes in carbon, nitrogen and C/N ratios in differently treated soil after 200 days*

Treatments	Nitrogen per cent			Carbon per cent			C/N ratio per cent		
	Original	Final		Original	Final		Original	Final	
		F	H		F	H		F	H
I. No manure . . . . .	0.049 (100)	0.050 (102.04)	0.049 (100)	0.73 (100)	0.67 (91.78)	0.71 (97.26)	14.9 (100)	13.4 (89.93)	14.5 (97.32)
II. Farmyard manure (0.33 per cent)	0.051 (100)	0.063 (123.53)	0.058 (113.73)	0.74 (100)	0.99 (133.78)	0.80 (108.11)	14.5 (100)	15.7 (108.28)	13.8 (95.17)
III. Farmyard manure (1 per cent)	0.054 (100)	0.068 (125.93)	0.058 (107.41)	0.78 (100)	1.15 (147.44)	0.84 (107.69)	14.4 (100)	16.9 (117.36)	14.5 (100.69)

It will be seen that the nitrogen contents of the control soil remain practically unchanged even after 200 days under both the moisture conditions. With the addition of farmyard manure (treatments II and III), however, there have been increases in nitrogen with both the doses of manure. Since the nitrogen-fixing organisms require energy-materials [Waksman, 1931], addition of farmyard manure is beneficial as it supplies all the necessary energy while undergoing oxidation in the soil. In the present experiment it is noticed that there is more gain in nitrogen at field saturation than at half-saturation moisture which suggests the possibility of anaerobic bacteria like *Clostridium pasteurianum* taking an active part in these soils.

With regard to carbon there is only a slight lowering in the values in the case of control soil, whereas in all the other treatments there is a gain. Increase in carbon is more pronounced at field saturation than at half saturation. With the addition of farmyard manure, the increases are 34.47 per cent at field-moisture saturation for the lower and higher doses of manuring respectively, while in the case of half saturation the increases are much smaller. The question of such increases in the carbon contents of soil due to manuring was further investigated in a separate experiment with different manure samples. This is described later.

Finally, as a result of lowering in carbon in the control soil, the C/N ratio decreases a little, especially at field-moisture saturation. In the case of farm-yard manuring, although there are increases in both carbon and nitrogen they are almost balanced in the case of half-saturation moisture but at field-moisture saturation there is an ultimate increase in the ratio in both the treatments.

### *Second series of experiments*

With regard to the fixation of carbon that was found to take place in the previous experiment, a separate investigation was conducted with different samples of manure on a second sample of farm soil having a C/N ratio of 14.35. The experiment was carried out in flat glass dishes with 200 gm. of soil with addition of 2 gm. of manure at 44 and 22 per cent moisture levels, respectively, for a period of one month. The soils were not stirred in this experiment and exposed to sunlight every day for one hour in order to encourage the algal growth if any. The results are given in Table VI.

TABLE VI

*Changes in carbon, nitrogen and C/N ratios in soil with application of different samples of farmyard manure at two moisture levels†*

Period	Farmyard manure No. 1* (6 months' old)						Farmyard manure No. 2* (12 months' old)					
	F			H			F			H		
	Carbon	Nitro- gen	C/N	Car- bon	Nitro- gen	C/N	Carbon	Nitro- gen	C/N	Carbon	Nitro- gen	C/N
Original.	0.84 (100)	0.058 (100)	14.5	0.84 (100)	0.058 (100)	14.5	0.80 (100)	0.057 (100)	14.2	0.80 (100)	0.057 (100)	14.2
A month after	0.88 (104.76)	0.064 (110.34)	13.62	0.84 (100)	0.062 (106.90)	13.5	0.87 (108.75)	0.056 (98.25)	15.4	0.82 (102.50)	0.066 (115.79)	12.4

Period	Compost No. 7* (8-10 months' old)						Farmyard manure No. 8* (2 year's old)					
	F			H			F			H		
	Carbon	Nitro- gen	C/N	Carbon	Nitro- gen	C/N	Carbon	Nitro- gen	C/N	Carbon	Nitro- gen	C/N
Original.	0.79 (100)	0.057 (100)	13.7	0.79 (100)	0.057 (100)	13.7	0.77 (100)	0.054 (100)	14.2	0.77 (100)	0.054 (100)	14.2
A month after.	0.80 (101.27)	0.063 (110.53)	12.7	0.76 (96.20)	0.065 (114.04)	11.7	1.02 (132.47)	0.063 (116.67)	16.10	0.81 (105.19)	0.059 (109.26)	13.7

\* These numbers refer to those given in Table II

† F = Field-moisture saturation ; H = Half-moisture saturation

It will be observed that the C/N ratios have gone down to a certain extent in all treatments with the exceptions of farmyard manures Nos. 2 and 8 where there are slight increases in the values at field-moisture saturation. Looking into the individual figures of carbon and nitrogen it will be noticed that in the case of soil treated with a fresh manure (No. 1, i.e. 6 months old) there is practically no increase of carbon but the gains in nitrogen are 10 and 7 per cent at

field and half saturation respectively. With manure No. 2 (1 year old, which was used also in the previous experiments) increase in carbon to about 9 per cent is noticed in field-saturation moisture and a gain in nitrogen of 16 per cent is noticed at half-saturation. Using compost prepared at the farm there is practically no increase in carbon under both the moisture conditions, whereas gains in nitrogen from 11 to 14 per cent are observed. In the case of soil treated with manure No. 8 (2 years old) there have been 32 per cent and 6 per cent increases in carbon in field and half saturation respectively, whereas gains in nitrogen are 17 and 9 per cent. This experiment shows definitely the varying nature of manure samples with regard to inducing fixation of carbon and nitrogen. As soil-algae are known to be capable of synthesizing complex organic substances from  $\text{CO}_2$  and water in sunlight [Russell, 1923], it is only natural to attribute this increase in carbon to this source. Further, recent experiments in the laboratory have demonstrated visible algal growth in soils treated only with manure Nos. 2 and 8 at field-moisture saturation.

### *Third series of experiments*

Two soils were chosen for this experiment, having fairly high C/N ratios but the carbon content of one was about three times that of the other. The idea of the experiment was to test whether, apart from the question of high ratio, the actual amounts of carbon and nitrogen affect the decomposition of manures in any way. Both these soils had the same history, namely, that cane was grown on them for a long time and the yield was failing off gradually during the past years. Both belonged to the same soil type (i.e. B type). One of the soils had a ratio of 22.5 (possessing both higher carbon and nitrogen contents) while the other had a ratio of 17.3, having lower carbon and nitrogen contents. (It is rather unfortunate that exactly the same ratio was not obtainable in these two soils having the same past history and belonging to the same soil type). The treatments were the same in both the soils, viz.—

I. Control soil—no manure.

II. Addition of farmyard manure\* corresponding to 1 per cent of soil used.

Soils were kept at half-moisture saturation by addition of water every 9th day when soil samples were also given a thorough stirring by hand. Detailed determinations were continued up to a period of 81 days. In the discussion that follows the soil having higher contents of both carbon and nitrogen will be referred to as No. 1 and the other as No. 2.

### *Nitrogen changes and bacterial numbers*

In Table VII, the mineral nitrogen contents at different periods for the two soils are given.

It will be observed that the mineral nitrogen contents of soil No. 1 are generally higher than those of soil No. 2 in the case of the untreated soils. The application of farmyard manure has not resulted in any increase in mineral nitrogen in the case of soil No. 1. On the contrary a slight lowering in the values is observed at the earlier stages. With soil No. 2, farmyard manure has given some additional mineral nitrogen over the control. These results

\* In this series the sample of farmyard manure used is the same as No. 2 of Table II

will be quite clear from Table VIII where the per cent mineralization figures at different periods are given. For the sake of comparison similar figures for the normal fertile soil already described under the first series of experiments are included.

TABLE VII

*Mineral nitrogen in mg. per 100 gm. of air-dry soil at half-moisture saturation for soil Nos. 1 and 2 with and without farmyard manure deteriorated soils*

Serial No.	Treatment	Days									
		0	9	18	27	36	45	54	63	72	81
1	No manure . . . . .	1.46	1.82	1.16	1.49	1.74	1.37	1.21	1.27	0.98	0.96
	Farmyard manure (1 per cent)	1.46	0.78	0.84	1.27	1.56	...	1.41	1.31	1.23	1.26
2	No manure . . . . .	1.12	1.14	0.72	1.53	0.97	0.85	0.86	1.02	0.90	0.71
	Farmyard manure (1 per cent)	1.12	1.08	1.26	1.60	1.22	1.81	1.03	1.19	1.16	0.82

TABLE VIII

*Periodic excess of mineral nitrogen over the control expressed as percentages of the added nitrogen in the form of farmyard manure to fertile and deteriorated soils at half-moisture saturation*

	Number of days from commencement						
	0	7	14	21	28	35	42
Normal fertile soil C/N = 14.9	0.00	12.72	7.71	7.31	7.71	1.62	28.96

	Number of days from commencement									
	0	9	18	27	36	45	54	63	72	81
Soil No. 1 C/N = 22.5	0.00	-14.07	-4.33	-2.98	-2.44	...	2.71	0.54	3.38	4.06
Soil No. 2 C/N = 17.3	0.00	-0.81	7.37	0.95	3.38	12.99	2.30	2.30	3.52	1.49

The poor responses of the farmyard manure in the deteriorated soils possessing higher C/N ratios are at once self-evident. Soil No. 1 in this respect is very much inferior to soil No. 2.

Referring now to Fig. 2 the bacterial numbers show very similar fluctuations in the case of both the soils except that at earlier stages soil No. 2 exhibits much lower numbers. Generally speaking, farmyard manuring has enhanced the bacterial activity in both the soils as was also observed with normal fertile soil at half-moisture saturation. The differences in the periodic fluctuations in the bacterial numbers and mineral nitrogen in the deteriorated and normal soils are worth observing (Fig. 1). The sudden release of mineral nitrogen is not observed in the case of the deteriorated soils when the

bacterial numbers attain low figures as in the case of the normal soil. This peculiar behaviour of the deteriorated soils requires further careful studies.

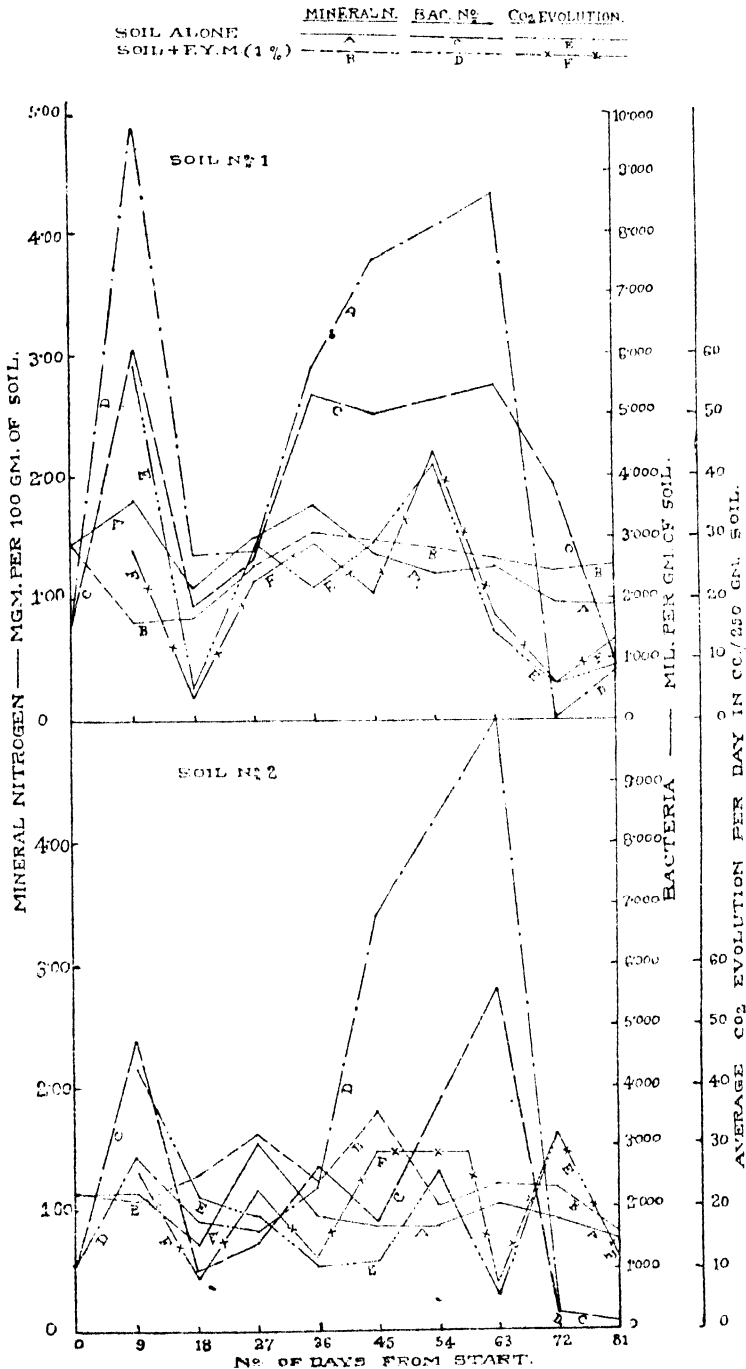


FIG. 2. Periodic fluctuations in mineral nitrogen, bacterial numbers and carbon dioxide evolution—deteriorated soils

### Respiration studies

Respiration studies were conducted on these two soils and the results are also shown in Fig. 2. The evolution of carbon dioxide from the control soils indicates, on the whole, better microbiological activities in soil No. 1 than in soil No. 2, which fact is also borne out by bacterial counts. It will be apparent that, in general, the periodicity of  $\text{CO}_2$ -evolution shows similar trends to bacterial activity in both the soils. The addition of farmyard manure has, however, shown an initial set-back in figures of  $\text{CO}_2$  in both the soils, while in soil No. 1 the average production is also lowered by manuring. It is, however, difficult to reconcile these opposite facts (i.e. increased bacterial activity and lowering in  $\text{CO}_2$ -evolution by farmyard manuring) unless formation of algae within the short period between stirring of the soils with the consequent absorption of  $\text{CO}_2$  is assumed.

### Changes in the soils after 327 days of alternate drying and wetting at half-moisture saturation

The process of wetting (to 50 per cent of the moisture-holding capacities) and drying of the soils in pots with regular periodic stirring was continued till 327 days to see the ultimate effect on the soil. The soil samples after this period were air-dried and total nitrogen and carbon determined. The results calculated on 100 gm. of oven-dry soil are given in Table IX, together with the C/N ratios and the per cent increases over the original in brackets.

TABLE IX

*Final changes in nitrogen, carbon and C/N ratios in differently treated soils after 327 days*

Soil No.	Treatment	Control			Farmyard manure (1 per cent)		
		N	C	C/N			C/N
1	Original . .	0.067 (100)	1.51 (100)	22.5 (100)	0.076 (100)	1.64 (100)	21.6 (100)
	Final . .	0.086 (128.36)	0.99 (65.56)	11.5 (51.11)	0.101 (132.89)	1.10 (67.07)	10.9 (50.46)
2	Original . .	0.030 (100)	0.52 (100)	17.3 (100)	0.040 (100)	0.65 (100)	16.3 (100)
	Final . .	0.049 (163.3)	0.66 (126.8)	13.9 (80.8)	0.060 (150.0)	0.52 (80.00)	8.7 (53.30)

Table IX shows that there are increases in nitrogen even in the control soil, the increase being more in soil No. 2 where the nitrogen content at the start was very low. It appears that, unlike the normal soil these soils with high C/N ratios are capable of fixing nitrogen even without addition of energy material from outside sources. With regard to carbon there is a loss in the case of soil No. 1 but in the case of soil No. 2 (where the original carbon was low) there is a slight gain. The ultimate result is, however, a lowering in the C/N ratio in both the soils. (This is more pronounced in soil No. 1 than in soil No. 2). With the addition of farmyard manure (energy material) there is fixation of nitrogen in both cases although the percentage increases

are not very much different from those observed in the control soil. Losses of carbon occur in both the soils and the resulting C/N ratios are much lower than the original values. Addition of farmyard manure appears to be more useful in lowering the ratio in the case of soil No. 2. The above experiment suggests the possibility of reclaiming soils having high C/N ratio by the simple process of wetting and drying with regular bulking of the soil (i.e. light cultivation operations) which may be further helped by the addition of farmyard manure in case the soil is poor in both carbon and nitrogen.

#### SUMMARY AND CONCLUSIONS

With reference to the poor response of sugarcane to application of farmyard manure which was observed in the experiments conducted at the Padegaon Farm, and also, in order to investigate the important question of the rôle of bulky manures in modifying soil fertility under continued cane growing, three series of experiments were carried out on an important genetic soil type belonging to the broad group of black cotton soils. The results are given below :

In the first series the biological responses were studied of a normal fertile soil obtained from an experimental block on the farm to the application of two doses of farmyard manure. Moisture was maintained at two levels in two different sets of pots, one at field saturation and the other at half saturation, approximating to conditions under perennial irrigation and dry farming, respectively.

In general, the mineral nitrogen contents of soils at field-moisture saturation was higher than those at half-moisture saturation. The application of farmyard manure, however, raised the mineral nitrogen status at half-moisture saturation, while it adversely affected the status at field saturation. Bacterial counts taken periodically showed lowered bacterial population as a result of manuring at field-moisture saturation, while reverse was the case at half-moisture saturation. Deleterious effect of field-moisture saturation on the mineralization of farmyard manure is thus indicated.

The changes in carbon and nitrogen status of soils after 200 days were interesting. Although no appreciable changes were noticed in the untreated soil, considerable increases, both in carbon and nitrogen, were observed at field moisture with the addition of farmyard manure, and the resulting C/N ratios were raised in both the doses of manuring. In the case of half-moisture although slight increases in these constituents were noticed, the ratios remained practically unaffected.

In order to find out whether this increase in carbon due to farmyard manure application is a general one, a second series of experiments was conducted with four samples of farmyard manure at two moisture levels without disturbing the soils. The results indicated the specific nature of manures in inducing fixation of carbon in soils under such conditions within a short period. Further experiments have demonstrated that the presence of algae in certain manure samples is responsible for this fixation by their development at field-moisture saturation. Thus, samples of manure which were found to contain no algae, were unable to increase the carbon contents of soils.

In a third series of experiments, two samples of soil where cane yields were falling and which showed high C/N ratios were taken for study. The

availability of nitrogen by the application of farmyard manure even at half-saturation moisture was found to be very poor in these soils when compared to a normal fertile soil having a lower C/N ratio. In spite of increased bacterial activity as a result of farmyard manuring in these soils, mineralization of nitrogen was found to be defective. This defective mineralization of farmyard manure in these soils requires further careful microbiological studies.

The final changes in the carbon and nitrogen status of these soils indicated the possibility of ameliorating these soils (by lowering the C/N ratios) by repeated wetting and drying in conjunction with regular bulking of the soils periodically.

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# STUDIES ON INDIAN RED SOILS

## V. FACTORS RESPONSIBLE FOR BUFFER CAPACITIES AND BASE-EXCHANGE PROPERTIES

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(With three text-figures)

**A**LL soils possess considerable but widely different buffer capacities, which means that these substances contain active material which tends to counteract the changes in the reaction brought about by the addition of basic or acidic substances. Besides, small amounts of dissolved buffering salts like phosphates, carbonates and salts of organic acids, both clay and humus, act as strong buffers.

Raychaudhuri and Nandymazumdar [1940] have shown that buffer curves of profile samples of Indian red soils indicate a more or less definite inflexion at pH 9.8 and frequently a second inflexion either at pH. 2.9 or at 4.6. It was felt desirable to examine closely the factors which are responsible for the inflexion of the buffer curves at these pH values. Profile samples of red soils of India, collected from Bihar, Orissa, Assam and parts of Bengal have been used in this work. The chief base-exchange properties of soils, including the pH, air-dry moisture, total exchangeable bases, degree of base saturation, buffer curves and other related properties were determined. For the determination of buffer curves the method devised by Schofield [1933], has been followed. It has been pointed out by Raychaudhuri [1941] that the study of buffer curves by Schofield's method yields very valuable information regarding the nature of soil types.

### EXPERIMENTAL

#### *Determination of pH*

The pH values at soil-water ratio 1 : 2.5 were determined by Kuhn's barium sulphate method using a Hellige colorimeter and also by the quinhydrone electrode method.

#### *Determination of carbonates*

The determination of carbonates were made with the help of Collin's calcimeter.

#### *Determinations of air-dry moisture*

The air-dry moisture was calculated from the loss in weight of 2—5 gm. of soil heated in an electric oven at a temperature of 105°C. for 24 hours.

### *Determination of buffer curves*

A weighed quantity of soil was taken in a 40-c.c. dried test tube fitted with an indiarubber stopper and 25 c.c. of the buffer solution were added. The rubber stopper was fitted and the test tube was clamped to a rotating shaker which was rotated with the help of an electric motor for a period of 16 hours. The test tubes were taken out of the shaker and allowed to stand for nearly two hours. A measured volume of the supernatant liquid was pipetted off and titrated with a standard HCl or lime solution, as the case may be. In the case of buffer solutions of pH 1.3, 2.9, and 4.6, the figures for the uptake were corrected for the percentage carbonate present in the soil wherever the soil contained measurable amounts of carbonate. 0.06 N organic acid (as mentioned in Table I) were prepared which were half-neutralized with lime. Table I shows the the results with one soil sample (113p.)\*

TABLE I

*Uptake of base by soil No. 113p from half-neutralized buffer solutions of acid concentrations 0.06N and 0.04N at different pH values*

Organic acid	pH	0.06N	0.04N
Mono-chloroacetic acid . . . . .	2.9	—3.7	—3.58
Acetic acid . . . . .	4.6	—0.66	—0.52
P-nitrophenol . . . . .	7.1	+1.27	+1.30
Phenol . . . . .	9.8	+6.90	+7.24

The results in Table I show that within the limits of experimental error the uptakes of base at the two acid concentrations of the buffer solutions are practically the same.

### *Determination of total exchangeable bases*

The total exchangeable bases of the soil were determined by the method of Williams [1929]. The observed figures of the exchangeable bases were corrected for the carbonate contents of the soils wherever the soils contained measurable amounts of carbonate. A correction was made for the exchangeable bases in the reagents employed.

### *Determination of saturation capacity at pH 7.0*

The saturation capacities at pH 7.0 were determined by the barium acetate-ammonium chloride method of Parker [1929].

### *Determination of organic carbon*

The organic carbon was determined by the modified method of Walkley [1935].

\*The pH values at half-neutralized points of the acids at these concentrations would be the same following the equation  $pH = pKa$  at the half-neutralized points of the acids.

*Electrodialysis of soils*

The soils were electrodialysed in a three-chambered electrodialysis vessel. The substances were kept in the middle chamber and electrodialysis was carried out until the liquid at the cathode was neutral.

## RESULTS AND DISCUSSION

*Exchangeable bases, saturation capacities and base saturation*

Table II shows the m. eq. of exchangeable bases ( $x$ ), saturation capacities ( $y$ ) and percentage base saturation ( $x/y \times 100$ ), calculated on oven-dry basis.

TABLE II

*Exchangeable bases in m. eq. per cent, saturation capacities in m. eq. per cent and per cent base saturation*  
(Calculated on oven-dry basis)

Locality	Soil No.	Depth	$x$	$y$	$x/y \times 100$	pH
Hathwara, Manbhum, Bihar	81p	0—1 ft. 6 in. .	3.58	6.95	51.51	4.9
	82p	1 ft. 6 in.—2 ft. 3 in.	4.33	8.24	52.55	5.6
	83p	2 ft. 3 in.—3 ft. 6 in.	5.91	8.80	67.16	5.8
	84p	3 ft. 6 in.—4 ft. 11 in.	5.16	8.85	58.31	5.9
	85p	4 ft. 11 in. below	5.29	8.45	62.60	5.8
Jhinkartangi, Khurda Town, Orissa	106p	0—1 ft. .	7.06	nd	..	4.5
	107p	1 ft.—2 ft. .	6.71	8.67	77.39	4.8
	108p	2 ft.—8 ft. 6 in.	8.26	10.95	75.44	5.3
	109p	8 ft. 6 in.—10 ft.	7.07	7.80	90.64	5.9
	110p	30 ft.—50 ft. .	4.17	4.49	97.33	6.8
Lalgarh, Midnapur, Bengal	112p	0—4 in. .	5.95	11.7	50.85	5.8
	113p	4 in.—3 ft. 4 in.	5.99	9.83	60.93	5.6
	114p	3 ft. 4 in.—4 ft.	4.98	7.19	69.26	5.3
	115p	7 ft.—8 ft. .	4.15	9.83	43.22	4.5
Cheerapunji, Khasi Hills, Assam	124p	0—7 in. .	1.33	2.9	55.65	4.0
	125p	7 in.—10 in. .	3.00	4.66	64.38	4.6
	126p	10 in.—4 ft. .	3.86	4.12	93.68	5.0
	127p	10 ft. below .	6.74	nd	..	7.2

The data in Table II show that in the case of Hathwara (Manbhum, Bihar), the percentages base-saturation increase down the profile and remain fairly constant. In the case of Jhinkartangi (Khurda Farm, Orissa), the percentages base-saturation remain fairly constant down the profile and then increase at greater depths. In the case of Lalgah (Midnapur, Bengal), the percentages base-saturation increase down the profile and then decrease at greater depth. In the case of soils of Cheerapunji (Khasi Hills, Assam), the  $x/y$  values increase regularly as the depth of the profile increases.

#### *Study of buffer curves*

Table III gives the data on the m. eq. of base taken up by 100 gm. of oven-dry soils at pH values 1.3, 2.9, 4.6, 7.1, 9.8 and 12.5.

TABLE III

*M. eq. of base taken up by 100 gm. of oven-dry soil at different pH values*

Locality	Soil No.	Depth	pH 1.3	pH 2.9	pH 4.6	pH 7.1	pH 9.8	pH 12.5
Hathwara, Manbhum, Bihar	81p	0—1 ft. 6in. .	—9.1	—4.1	—0.59	1.4	9.8	13.7
	82p	1 ft. 6in.—2ft. 3 in.	—8.2	—4.8	—1.2	0.91	11.5	17.0
	83p	2 ft. 3in.—3ft. 6 in.	—8.7	—5.0	—1.4	0.90	11.8	21.5
	84p	3 ft. 6in.—4ft. 11 in.	—7.4	—4.8	—1.0	0.90	10.6	12.9
	85p	4 ft. 11 in. below	—7.6	—2.9	—0.71	0.69	5.1	13.4
Jhinkartangi, Khurda Town, Puri, Orissa	106p	0—1 ft. .	—25.8	—5.2	—0.07	7.7	25.6	43.7
	107p	1 ft.—2 ft. .	—10.7	—7.0	—0.56	4.9	22.3	34.4
	108p	2 ft.—8 ft. 6 in.	—10.7	—6.9	—1.4	3.6	23.5	42.0
	109p	8 ft. 6 in.—10 ft.	—11.4	—7.6	—1.7	2.5	21.3	25.7
	110p	From the diggings of a well	—11.1	—2.8	—1.4	0.13	4.9	5.4
Lalgah, Midnapur, Bengal	112p	0—4 in. .	—11.0	—5.8	—1.1	1.8	12.0	22.4
	113p	4 in.—3ft. 4in.	—8.0	—3.7	—0.66	1.3	6.9	17.6
	114p	3 ft. 4in.—4ft.	—7.0	—1.9	—0.18	0.89	4.1	14.1
	115p	7 ft.—8 ft. .	—6.9	—1.6	—0.11	1.9	7.5	14.9
Cheerapunji, Khasi Hills, Assam	124p	0—7 in. .	—2.2	—0.82	0.18	1.8	6.9	12.5
	125p	7 in.—10 in..	—6.4	—3.6	—0.47	3.3	11.1	18.6
	126p	10 in.—4 ft..	—6.9	—3.8	—1.4	1.7	7.6	17.9
	127p	10 ft. below .	—8.9	—5.7	—4.2	1.0	0.64	1.3

TABLE III - *contd.*

Locality	Soil No.	Depth	pH 1·3	pH 2·9	pH 4·6	pH 7·1	pH 9·8	pH 12·5
Nongpoh, Khasi and Jaintia Hills, Assam	134p	0—6 in.	9·3	5·5	0·26	9·4	32·3	39·7
	135p	6 in.—3ft. 6in.	8·3	4·1	0·48	14·1	33·8	44·0
	136p	3 ft. 6 in.—4 ft. 2 in.	5·5	2·3	2·0	15·5	39·9	58·2
	137p	4 ft. 2 in.—6 ft.	6·6	4·3	0·19	8·9	31·9	44·7
Uzanbazar, Gau- hati, Assam	139p	0—6 in.	10·8	6·3	1·1	3·4	17·4	26·9
	140p	6 in.—11 ft.	7·2	4·1	0·18	4·7	15·2	25·6
	141p	11 ft.—16 ft.	5·8	2·3	0·83	4·6	13·2	30·5
	142p	16 ft. below	4·3	1·8	0·18	1·1	5·7	8·0
	143p	From a cut- ting on the top of a hillock	7·0	2·4	0·84	5·0	16·2	31·9
Babupura, Tura, Garo Hills, Assam	152p	0—10 in.	10·8	6·6	0·37	9·5	33·0	39·5
	153p	10 in.—2 ft.	9·2	5·4	3·6	18·2	44·2	52·8
	154p	2 ft.—4 ft.	12·1	8·4	2·5	15·7	52·2	61·7
Sultanganj, Bogra, Bengal	156p	0—1 ft.	11·8	4·8	0·37	2·4	13·7	22·2
	157p	1 ft.—2 ft.	8·7	4·9	0·37	2·3	15·2	27·8
	158p	2 ft.—4 ft.	11·5	5·6	1·1	2·7	17·1	22·5
	159p	12 ft.—25 ft.	13·8	5·4	0·73	1·1	8·8	14·6
	160p	25 ft.—30 ft.	14·9	4·5	0·91	1·1	7·4	13·3
Khetur Road, Barind Tract, Rajshahi, Bengal	162p	0—1 ft. 10 in.	9·1	5·0	0·37	1·5	10·0	17·4
	163p	1 ft. 10 in.— 2 ft. 3 in.	12·6	6·2	0·75	1·7	15·0	25·3
	164p	2 ft. 3 in.—4 ft.	15·4	7·8	1·7	0·74	13·4	26·2

Table III shows that for soil samples 81p-85p (Hathwara Farm, Manbhum, Bihar) between pH ranges 1·3 to 7·1, the buffer capacities of all the soil samples are almost equal, but beyond pH 7·1, 85p (4 ft. 11 in. below) has least buffer action, whilst with soils 81p-83p, the buffer action increases as the depth of the soil sample in the profile layer increases. Soil No. 84p (3 ft. 6 in.—4 ft. 11 in.) is intermediate in buffer capacity.

For soil samples 106p-110p (Jhinkartangi, Khurda Farm, Puri, Orissa), the buffer capacities in general decrease as we pass from the top layer downwards. At higher pH regions 108p possesses greater buffer action than 107p. With samples 112p-115p (Lalgarh, Midnapur, Bengal), the buffer capacities decrease as the depths of the profile increase. With soil samples 124p-127p (Cheerapunji, Khasi Hills, Assam), the top soil 124p has moderate buffer capacity whilst soils of intermediate layers 125p (7—10 in.) and 126p (10 in.—4 ft.) possess greater buffer action than top soil. Soil of the lowest layer of the profile 127p (10 in. below) possesses least buffer action. With samples 134p-137p (Nongpoh, Khasi and Jaintia Hills, Assam), the top soil (134p) is found to possess the least buffer action, whilst the soil from third layer, 136p (3 ft. 6 in.—4 ft. 2 in.), has got maximum buffer action. Soil No. 137p (4 ft. 2 in.—6 ft.) has got almost the same buffer action as that of 135p. The buffer capacities of soil samples 139p-141p (Uzanbazar, Gauhati, Assam) are almost equal, 142p of the same profile being an exception. With soil samples 152p—154p (Babupura, Tura, Garo Hills, Assam), 152p (10 in.) has got least buffer action, whilst 154p (2 ft.—4 ft.) has got the maximum buffer action. With soil samples 156p—160p (Sultanganj, Bogra, Bengal), it is found that soil samples from intermediate layers 157p (1 ft.—2 ft.) and 158p (2 ft.—4 ft.) possess greater buffer action, whilst soil samples from bottom layers 159p (12 ft.—25 ft.) and 160p (25 ft.—30 ft.) possess less buffer action. With soil samples 162p—164p (Khetur Road, Barind Tract, Rajshahi, Bengal), the top soil samples 162p (0—1 ft. 10 in.) has got the least buffer action, whilst soil samples from 2nd and 3rd layers of the profile, 163p (1 ft. 10 in.—2 ft. 3 in.) and 164p (2 ft. 3 in.—4 ft.), possess almost equal buffer capacities.

The buffer action is due to buffer materials present in the soil and the mode of variation of buffer capacity shows the manner of accumulation of the buffering materials at different layers of the profiles. Within certain degree of approximations, the soil profiles studied can be classified from the mode of variations of buffer capacities at different layers of the profiles into the following four classes :

(1) Cases where the buffer action in general increases down the profile, e.g. profiles of Tura (Garo Hills, Assam) and Rajshahi (Bengal).

(2) Cases where the buffer action in general decreases down the profile, e.g. profiles of Khurda Road (Orissa), and of Midnapur (Bengal).

(3) Cases where the buffer action in general remains constant down the profiles, e.g. profile of Gauhati (Assam).

(4) Cases where the buffer action shows a maximum value at an intermediate depth of the profile, e.g. the profiles of Cheerapunji and Nongpoh (Assam) and of Bogra (Bengal).

In agreement with the observations made by Raychaudhuri and Nandymazumdar [1939], it is found that almost all the buffer curves\* indicate more or less definite inflexions at pH 9.8 and frequently a second inflexion either at pH 2.9 or at pH 4.6.

The variations of the buffer values ( $dB/dpH$ ) of the soil samples at these pH values [Van Slyke, 1922] do not show any regularity.

\*Not shown in figures

*Comparison of pH values of soils obtained by different methods*

Table IV summarizes the data on the pH values of soils obtained by Kuhn's method, by Quinhydrone electrode and from the intersection of buffer curves with the line of zero uptake of base.

TABLE IV

*Comparison of pH values of soils obtained by different methods*

Locality	Soil No.	Depth	pH by		
			Kuhn's method	Quinhydrone electrode	Buffer curve
Hathwara, Bihar	Maubhum,	81p 0—1 ft. 6 in.	4.9	5.55	5.65
		82p 1 ft. 6 in.—2 ft. 3 in.	5.6	5.71	5.75
		83p 2 ft. 3 in.—3 ft. 6 in.	5.8	5.65	5.75
		84p 3 ft. 6 in.—4 ft. 11 in.	5.9	5.81	6.35
		85p 4 ft. 11 in. below	5.8	6.16	5.6
Jhinkartangi, Town, Puri, Orissa	Khurda	106p 0—1 ft.	4.5	nd	4.55
		107p 1 ft.—2 ft.	4.8	5.92	4.75
		108p 2 ft.—8 ft. 6 in.	5.3	5.95	5.25
		109p 8 ft. 6 in.—10 ft.	5.9	6.18	5.55
		110p From the digging of a well	6.8	6.58	6.70
Lalgah, Bengal	Midnapur,	112p 0—4 in.	5.8	6.41	5.15
		113p 4 in.—3 ft. 4 in.	5.6	5.81	5.6
		114p 3 ft. 4 in.—4 ft.	5.3	5.96	5.7
		115p 7 ft.—8 ft.	4.5	5.01	5.25
Cheerapunji, Assam	Khasi Hills,	124p 0—7 in.	4.8	5.24	3.75
		125p 7 in.—10 in.	4.7	5.21	4.9
		126p 10 in.—4 ft.	5.0	5.18	6.05
		127p 10 ft. below	7.2	nd	8.2

TABLE IV—*contd.*

Locality	Soil No.	Depth	pH by		
			Kuhn's method	Quinhydrone electrode	Buffer curve
Nongpoh, Khasi and Jaintia Hills, Assam	134p	0—6 in.	4·9	4·84	4·8
	135p	6 in.—3 ft. 6 in.	4·7	5·06	4·4
	136p	3 ft. 6 in.—4 ft. 2 in.	4·4	4·59	3·8
	137p	4 ft. 2 in.—6 ft.	4·5	4·55	4·7
Uzanbazar, Gauhati, Assam	139p	0—6 in.	5·2	5·79	5·1
	140p	6 in.—11 ft.	4·8	5·29	4·7
	141p	11 ft.—16 ft.	4·8	5·22	4·1
	142p	16 ft. below	5·0	5·19	5·7
	143p	From a cutting on the top of a hillock	4·7	4·83	4·1
Babupara, Tura, Garo Hills, Assam	152p	0—10 in.	4·8	5·09	4·7
	153p	10 in.—2 ft.	4·6	4·91	4·3
	154p	2 ft.—4 ft.	4·7	5·29	4·1
Sultanganj, Bogra, Bengal	156p	0—1 ft.	5·6	6·55	5·6
	157p	1 ft.—2 ft.	5·5	6·72	5·2
	158p	2 ft.—4 ft.	5·5	6·32	5·6
	159p	12 ft.—25 ft.	5·7	6·58	6·0
	160p	25 ft.—30 ft.	5·8	6·52	6·2
Khetur Road, Barind Tract, Rajshahi, Bengal	162p	0—1 ft. 10 in.	5·6	6·78	6·0
	163p	1 ft. 10 in.—2 ft. 3 in.	5·6	6·22	6·1
	164p	2 ft. 3 in.—4 ft.	6·2	6·52	6·4

From Mattson's [1937] point of view the pH at which the buffer curves intersect the line of zero-adsorption should correspond to the 'equi-ionic

point'. The results in general show that the  $pH$  values obtained by the Kuhn's method and in general those indicated by the point of intersection of the buffer curves with the line of zero uptake of base are both slightly lower than those obtained by the quinhydrone electrode method. In general, the point of intersection of buffer curves with the line of zero uptake of base corresponds more closely with the  $pH$  values obtained by the Kuhn's method than with the quinhydrone  $pH$ .

#### *Buffer curves of minerals*

During the course of the present work it was felt desirable to compare the values for the uptake of base by the profile samples at different  $pH$  values with the corresponding uptake by some naturally occurring minerals like limonite, bauxite, halloysite, kaolin and montmorillonite (Table V).

TABLE V

*Uptake of base in m. eq. per 100 gm. of minerals at different pH values*

Minerals	$pH$ 1.3	$pH$ 2.9	$pH$ 4.6	$pH$ 7.1	$pH$ 9.8	$pH$ 12.5
Bauxite .	—0.94	—0.51	—0.26	+2.2	+8.3	+18.0
Halloysite .	—8.5	—3.6	—2.6	+0.38	+9.5	+34.3
Kaolin .	0.00	+4.2	+4.2	+4.5	+7.0	+18.0
Limonite .	—1.5	—1.8	—3.1	—0.88	+7.5	+11.8
Montmorillonite	—5.1	—1.7	—1.5	+0.38	+4.5	+10.5

Fig. 1 shows the nature of the buffer curves obtained. The curves show that the mineral halloysite possesses higher buffer capacities than kaolin. All the curves are typically S-shaped ones, and show inflexions at  $pH$  2.9. The mineral limonite shows an interesting behaviour in that, contrary to expectations, the uptake of base at  $pH$  4.6 by this substance is higher than the uptake of base at  $pH$  2.9. This is probably due to complex sparingly soluble iron compounds being formed whose solubility varies differently with variation in  $pH$  values.

#### *Buffer curves of Merck's humic acid*

The results are shown in Table VI.

TABLE VI

*Uptake of base in m. eq. per 100 gm. of humic acid at different pH values*

	$pH$ 1.3	$pH$ 2.9	$pH$ 4.6	$pH$ 7.1	$pH$ 9.8	$pH$ 12.5
Humic acid .	—39.7	—27.9	+53.7	+123.6	+268.3	+406.9

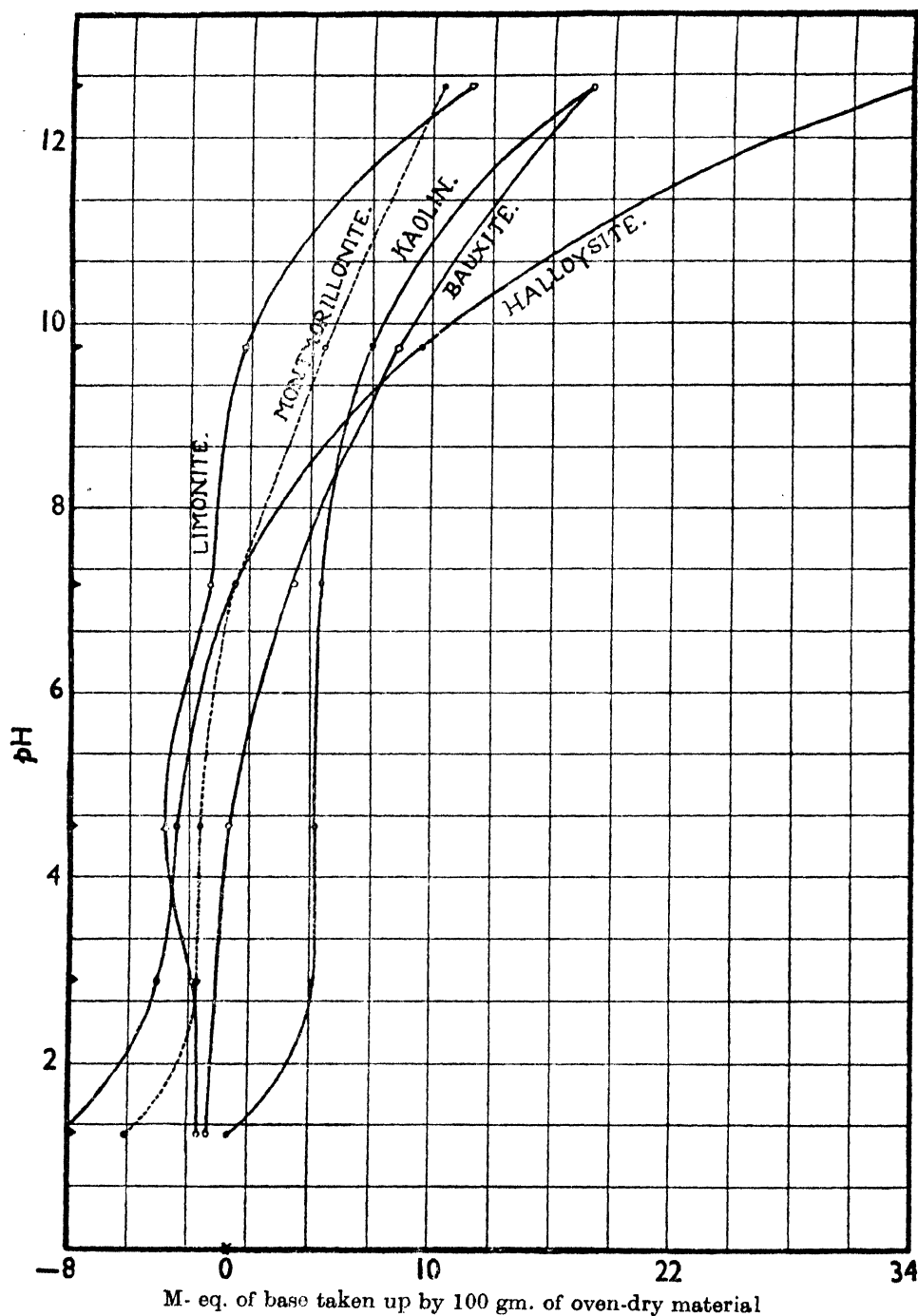


FIG. 1

The results are plotted graphically in Fig. 2. The curve shows an inflexion at pH 4.6. Raychaudhuri and Nandymazumdar [1939] previously suggested that the inflexion of buffer curves in this region might be due to the

presence of free alumina, since at about pH 4.6, alumina tends to go into colloidal state. The present observation, however, suggests that this in-

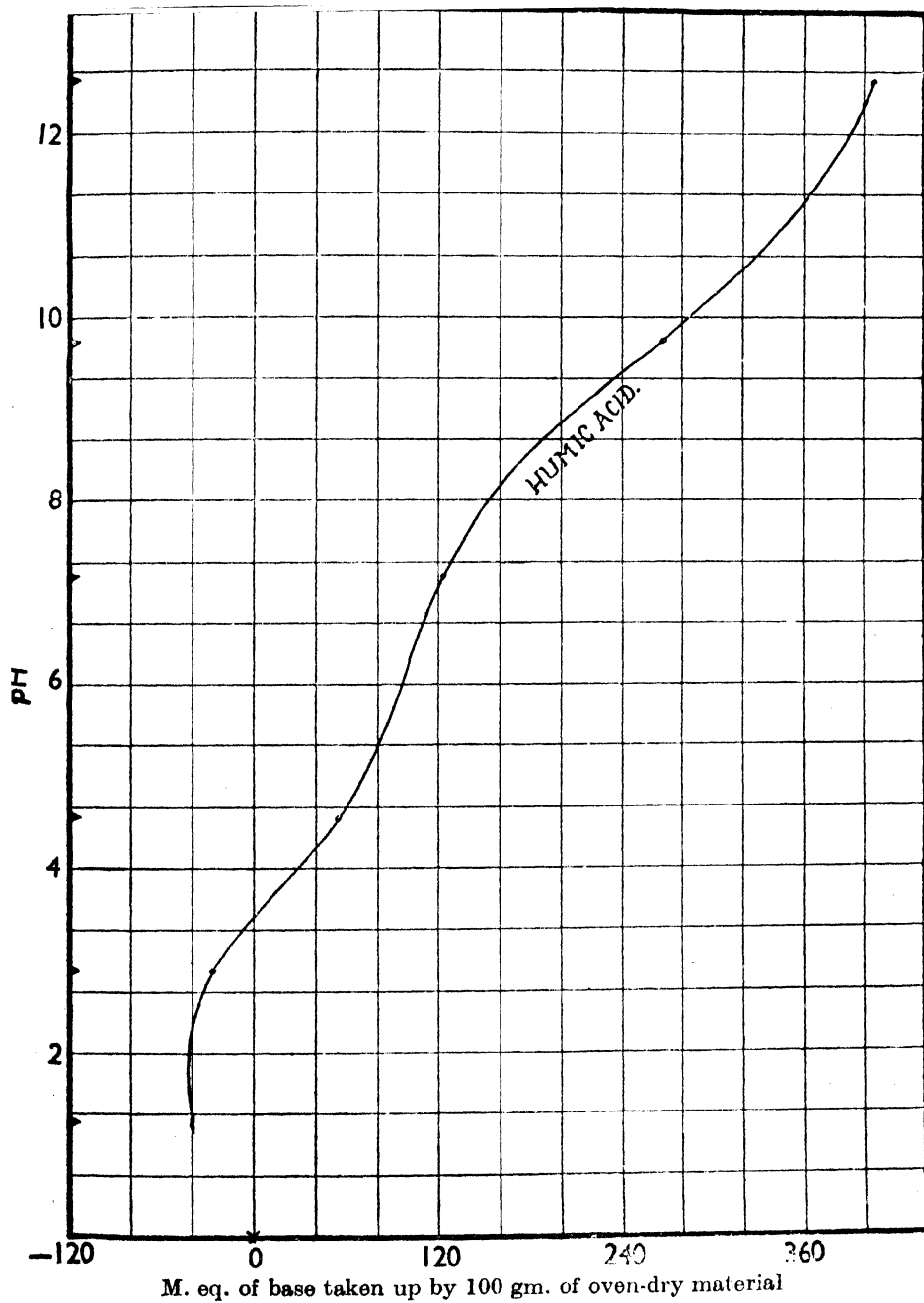


FIG. 2

flexion at about pH 4.6 might be due, at least to some extent, to organic matter which is present in the soil. This point requires further detailed investigations.

*Comparison of buffer curves by using different alkalies as the adsorbed base*

In the course of the investigation on the inflexion points of the buffer curves, drawn with calcium buffers at the three pH values—2.9, 4.6, and 9.8—it was thought that the solubilities of the resulting calcium silicates might have something to do with the inflexions of the curves.

The adsorption of base, according to Wiegner [1931], depends on the hydration of the cation, whilst according to the point of view of Mukherjee [1922], the adsorbability of cation would be determined by its valency and its electrical mobility. According to these hypotheses we would expect the buffer capacity of the soil to follow the order of lyotropic series, that is  $\text{Ca} > \text{K} > \text{Na} > \text{Li}$ .

Table VII gives the data on the relative adsorbabilities of the cations Ca and Na for three air-dry soils (85p, 113p and 124p), at different pH values.

TABLE VII

*Uptake of calcium and sodium [as  $\text{Ca}(\text{OH})_2$  and  $\text{NaOH}$ ] by three soils at different pH values*

pH		85p	113p	124p
1.3 . . . . .	{ Ca	—7.6	—8.0	—2.2
	{ Na	—7.0	—7.2	—2.0
2.9 . . . . .	{ Ca	—2.9	—3.7	—8.2
	{ Na	—3.1	—4.7	—1.5
4.6 . . . . .	{ Ca	—0.71	—0.66	0.18
	{ Na	—0.22	—0.74	—0.51
7.1 . . . . .	{ Ca	0.69	1.3	1.75
	{ Na	0.22	0.68	1.58
9.8 . . . . .	{ Ca	5.11	6.9	6.9
	{ Na	3.88	5.0	4.0

In general the data in Table VII uphold Mukherjee's [1922] theory of ionic adsorption, in that the adsorption of Ca is greater than that of sodium. A few exceptions are, however, noticeable. For instance, in the case of pH 1.3, the negative adsorption of Na is greater than that of calcium, which means that positive adsorption of sodium is higher. Another exception is shown by the relative adsorption of two bases at pH 4.6 in the case of soil No. 85p.

*Comparison of bases taken up by soils at different pH values*

Table VIII gives the data on the uptake of m. eq. of different bases by 100 gm. of air-dry soil (No. 113p), the results being expressed on oven-dry basis as usual.

TABLE VIII

*M. eq. base taken up by 100 gm. of oven-dry soil No. 113p*

pH	Li	Na	K	Ca
1.3	—8.3	—7.2	—7.0	—8.0
2.9	—4.5	—3.7	—3.2	—3.1
4.6	—1.6	—0.74	—0.72	—0.66
7.1	0.65	0.68	0.81	1.27
9.8	5.7	5.0	6.2	6.9
12.5 (Baryta)	17.6	17.6	17.6	17.6

Table IX, on the other hand, gives the data on the uptake of base by 100 gm. of air-dry electrodialysed soil (No. 113p), results being expressed on oven-dry basis. In the case of electrodialysed soil it was found necessary to centrifuge the mixture in order to get a clear supernatant liquid. The percentage of moisture in the electrodialysed soil was 2.93.

TABLE IX

*M. eq. of base taken up by 100 gm. of electrodialysed soil No. 113p*

pH	Li	Na	K	Ca
1.3	—3.5	—3.4	—3.3	—3.2
2.9	—1.32	—1.34	0.4	1.3
4.6	—0.38	2.6	3.3	0.4
7.1	8.4	8.5	8.7	9.6
9.8	12.9	14.5	15.0	16.1
12.5 (Baryta)	28.8	28.8	28.8	28.8

The data in Tables VIII and IX show that the adsorption of metal cations are generally in the order  $\text{Ca} > \text{K} > \text{Na} > \text{Li}$ . Ca is divalent and hence it should be adsorbed to the maximum extent by the negatively charged clay. The adsorption of K, Na and Li follows the order of solvation of these ions and hence is in agreement with the lyotropic series. There is, however, one peculiarity to be noticed in the relative order of curves. It will be found that the relative difference in the adsorption of different cations are maximum at pH 4.6, specially for the electrodialysed soil. The relative difference between the adsorbabilities by the different cations are fairly considerable at pH 9.8, whilst between pH ranges 4.6 to 1.3, the difference between the adsorbabilities narrows down to zero. Also at pH 7.1, the adsorbabilities of different cations are practically the same.

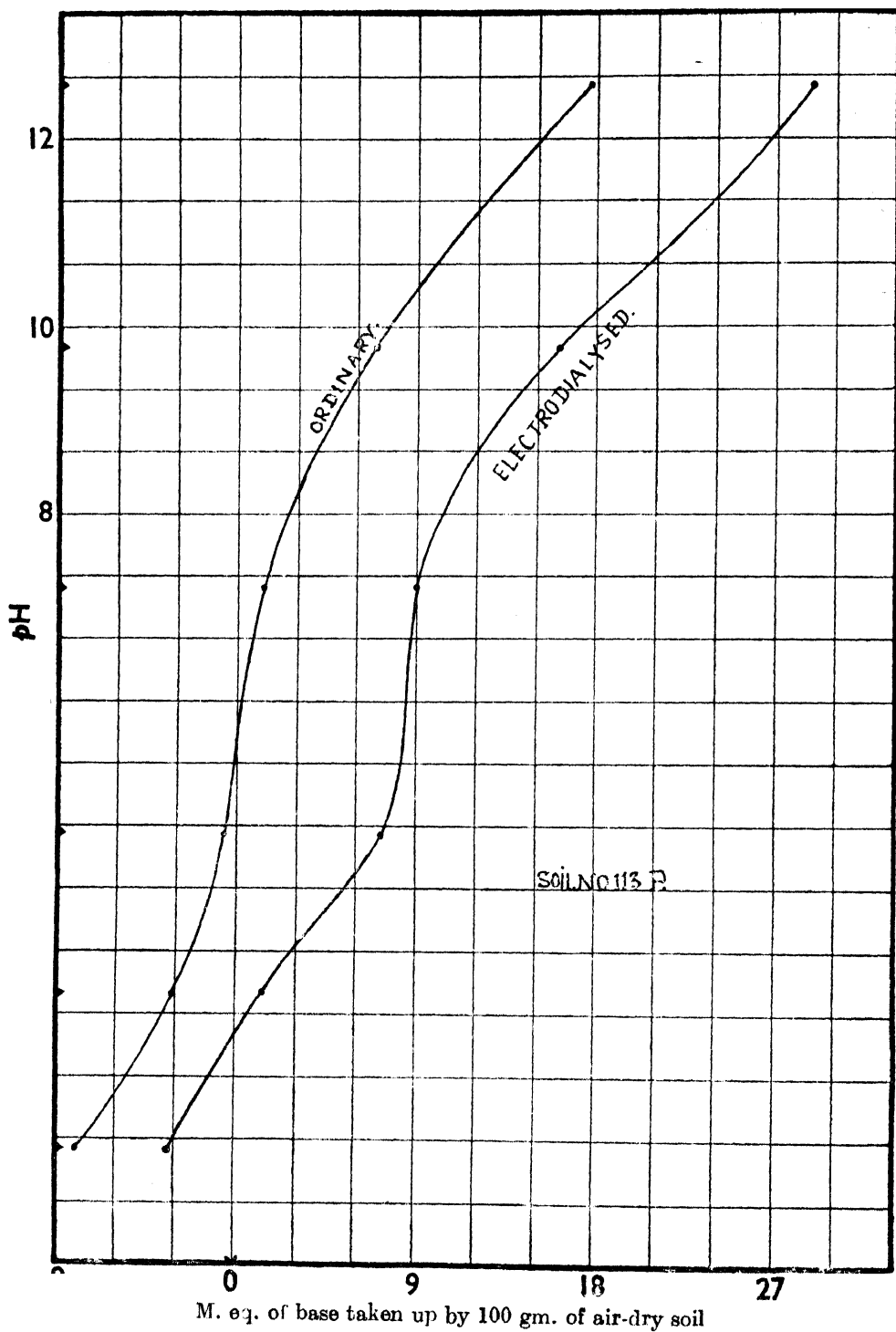


FIG. 3

*Uptake of calcium under different conditions*

Table X shows the results on the uptake of lime by two soil samples 113p and 152p before and after electro dialysis. The results with soil 113p before and after electro dialysis are plotted graphically in Fig. 3. The buffer curves of 152 p before and after electro dialysis show the same general behaviour.

TABLE X

*Uptake of lime by soil samples 113p and 152p before and after electro dialysis is*

pH	113p		152p	
	Ordinary air-dry soil	Electrodialysed soil	Ordinary air-dry soil	Electrodialysed soil
1·3	—8·0	—3·2	—10·8	—3·20
2·9	—3·1	1·3	—6·6	0·53
4·6	—0·66	7·4	—0·37	5·6
7·1	1·3	9·0	9·5	18·3
9·8	6·9	16·1	33·0	37·3
12·5	17·6	28·8	39·5	52·8

The percentage of moisture of the electro dialysed soil 113p was 2·93 and that of the electro dialysed soil 152p was 3·83. As is to be expected, the buffer curves of the ordinary air-dry soil and of the same soil after it has been electro dialysed run almost parallel to each other, the reason being that electro dialysis of soils causes replacement of ordinary exchangeable bases by hydrogen.

## SUMMARY AND CONCLUSIONS

In the present paper the chief base-exchange properties of a number of profile samples of red and lateritic soils of India have been studied. This includes the study of pH, percentages of air-dry moisture, total exchangeable bases, degree of saturation and buffer curves. Buffer curves of minerals like bauxite, limonite, halloysite, kaolin, montmorillonite and of Merck's humic acid have been studied. The relative adsorbabilities of cations calcium and sodium by the soils and the milli-equivalents of bases Li, Na, K and Ca taken up at different pH values have also been determined. Lastly, the determination of percentages of organic carbon of a number of soils and its influence on the nature of buffer curves have been studied. The main general conclusions are :—

1. Percentages of base-saturation of the profile samples generally increase down the profile. In some cases the percentage base-saturation attains a maximum at an intermediate depth.

2. Within certain degree of approximation the soil profiles studied can be classified from the mode of variation of buffer capacities at different layers of the profiles into four classes.

3. Almost all the buffer curves indicate more or less definite inflexion at  $pH$  9.8 and frequently a second inflexion either at  $pH$  2.9 or at  $pH$  4.6.

4. A comparison was made of the  $pH$  values obtained by the Kuhn's method, the quinhydrone electrode and that obtained from the intersection of the buffer curves with the line of zero uptake of bases. In general, the points of intersection of the buffer curves with the line of zero uptake of base corresponds very closely with the  $pH$  values obtained by Kuhn's method. Both these  $pH$  values are slightly lower than those obtained by quinhydrone electrode method.

5. The nature of the buffer curves obtained with limonite, bauxite, halloysite, kaolin and montmorillonite shows that the mineral halloysite possesses higher buffer capacities than kaolin. All the curves are typical S-shaped ones and show inflexions at  $pH$  2.9. The mineral limonite shows an interesting behaviour, in that contrary to expectations the uptake of base at  $pH$  4.6 by this substance is higher than the uptake of base at  $pH$  2.9.

6. Humic acid shows a very high buffer capacity, and the buffer curve of the humic acid shows an inflexion at  $pH$  4.6.

7. The relative adsorbabilities of the cations calcium and sodium by three air-dry soils (85p, 113p and 124p) at different  $pH$  values have been compared. The adsorption of calcium is greater than that of sodium.

8. The buffer curves of ordinary air-dry soil 113p and of the same soil after electrodialysis with the four bases Ca, Li, Na and K are nearly coincident, and the lyotropic series holds good in practically all the cases.

9. The buffer curves of ordinary air-dry soil and the same soil after it has been electrodialysed run almost parallel to each other.

#### ACKNOWLEDGEMENTS

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# STUDIES ON THE CHEMICAL CONSTITUENTS OF INDIAN LATERITIC AND RED SOILS

## III. DETERMINATION OF THE PERCENTAGE OF CLAY, MAXIMUM WATER-HOLDING CAPACITY AND OF FREE IRON OXIDE, FREE ALUMINA AND FREE SILICA OF LOWERMOST LAYERS OF PROFILE SAMPLES

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(With two text-figures)

RAYCHAUDHURI and Sulaiman [1940] have laid stress on the importance of determining the percentages of free silica and of free alumina and of free iron oxide in the case of lateritic soils. These authors have determined the percentages of free sesquioxides in Indian lateritic and red soils following the methods devised by Hardy [1931] and by Drosdoff and Truog [1935]. The percentages of free iron oxides obtained by Hardy's method were found to be much smaller than those obtained by the method of Drosdoff and Truog, whilst the percentages of free alumina obtained by Hardy's procedure are much higher than those obtained by the other method. More recently Hardy [1939] and Truog [1936] have modified their previous methods. The unpublished work of Sulaiman shows that the percentages of free alumina obtained by the modified methods of Hardy and of Truog are very nearly equal. Of these two methods, however, the method devised by Truog and coworkers has got special advantage in that it is possible, by this method, to obtain soil residues free from iron and aluminium oxides. Raychaudhuri, Sulaiman and Basuraychaudhuri [1941], in a recently published work, have shown that the presence of free sesquioxides and free silica in Indian red soils has considerable influence on the buffer capacities of the soils in that the buffer curves of the residues after removal of the free silica and free sesquioxide components become steeper as compared to those of the corresponding original soils.

In connection with the work on lateritic and red soils of India, it was felt desirable to examine the physico-chemical properties of soil samples of lowermost layers of profiles of these soil types and find out correlation, if any, between the physico-chemical properties of soils from bottom layers of these profiles with the nature and quantities of the mineral assemblages present in the soils and the parent material, the fundamental assumption being that the lowermost layer could be least affected by weathering agencies [Bonnett, 1939].

## EXPERIMENTAL

The percentages of clay in the soil samples were determined by the method of Robinson [1933]. The maximum water-holding capacities of the samples were determined by following essentially the procedure devised by Keen and Raczkowski [1921]. The percentages of amorphous products of weathering, e.g. free  $\text{Fe}_2\text{O}_3$ , free  $\text{Al}_2\text{O}_3$  and free  $\text{SiO}_2$ , were determined by the method of Drosdoff and Truog [1935], subsequently modified by Truog and coworkers [1937]. The percentages of ferro-magnesian minerals present in the soils were determined by following the procedure suggested by Hendrick and Newlands [1923]. The results are shown in Table I where the percentages of the clay fraction in the soil and the percentages by volume of ferro-magnesian minerals (determined by the procedure used by Jeffries [1937]) are also included.

TABLE I

*Percentages of clay fractions, of maximum water-holding capacities, of free  $\text{Fe}_2\text{O}_3$  of free  $\text{Al}_2\text{O}_3$  and of free  $\text{SiO}_2$  of lowermost layer of the profile samples*

Locality	Depth	Soil No.	Per cent clay fraction	Per cent max. water-holding capacity	Per cent free $\text{Fe}_2\text{O}_3$	Per cent free $\text{Al}_2\text{O}_3$	Per cent free $\text{SiO}_2$	Per cent by volume of ferro-magnesian minerals
Hathwara, Manbhum, Bihar	4 ft. 11-in. below	85 p	24.2	58.2	2.86	0.53	0.550	30.0
Putida, Singbhum, Bihar	2 ft. 9 in.—4 ft.	89 p	20.6	61.9	6.49	1.01	1.095	8.0
Baralota, Daltonganj, Bihar	4 ft.—5 ft.	97 p	12.4	57.0	1.07	0.31	0.924	30.0
Tangi, Cuttack, Orissa	2 ft.—4 ft.	100 p	18.1	49.9	11.68	2.38	1.128	8.0
Dhanmandal, Cuttack, Orissa	5 in.—4 ft.	102 p	67.10	72.0	9.86	2.22	0.624	24.0
Kapileswar, Bhubaneswar, Orissa	2 ft. 11 in.—4 ft.	104 p	28.3	50.0	10.91	1.48	0.835	2.0
Jhinkar, Khurda, Orissa	8 ft. 6 in.—10 ft.	109 p	23.0	n. d.	8.38	1.80	0.780	3.0
Lalgarh, Midnapore, Bengal	3 ft. 4 in.—4 ft.	114 p	27.6	54.9	3.93	1.34	0.645	25.0
Midnapore (Bed of Cossye river)	Bed of Cossye river	117 p	48.4	67.0	1.06	0.42	0.775	28.0
Malda, Midnapore	40 ft. below	118 p	27.6	66.1	4.50	0.61	0.616	28.0
Mawphlang, Khasi Hills, Assam	2 ft. 11 in.—4 ft.	122 p	37.0	71.3	10.32	1.57	0.577	22.0
Upper Chandmari, Tura, Garo Hills, Assam	2 ft. 8 in.—4 ft.	147 p	8.20	50.2	1.46	0.49	0.779	×

Fig. 1 shows graphically the correlation between the percentages of the clay fractions of the soil samples and their maximum water-holding capacities. It is found that there is fair linear relationship between the two. A particularly

interesting case is that with sample 122 p (Mawphlang, Khasi Hills, Assam), where the maximum water-holding capacity is as high as 71.3, whereas the percentage of clay fraction is only 37.0. Similarly also, the sample 118 p shows a comparatively high value for maximum water-holding capacity (66.1), whilst the percentage of clay fraction is as low as 27.6.

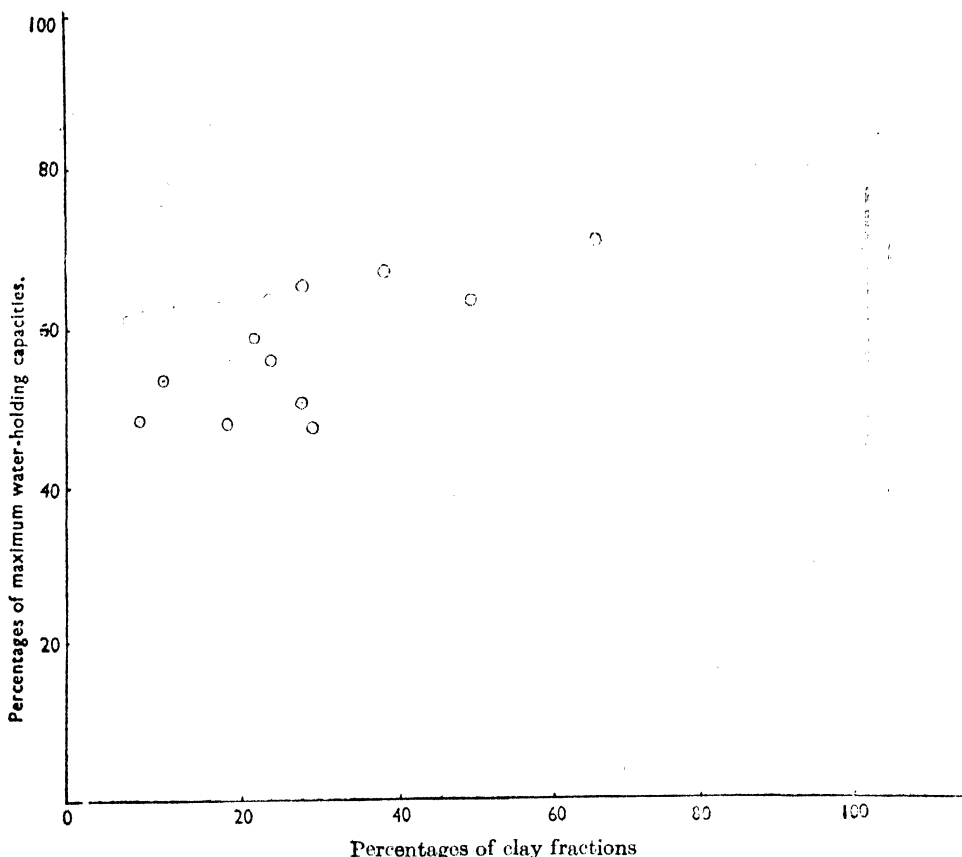


FIG. 1. Correlation between the percentages of clay fractions and their maximum water-holding capacities

Fig. 2 was drawn to show the relationship between the percentages of free alumina and of free iron oxide, with the percentages of ferro-magnesian minerals. It is generally to be expected that the greater the proportion of ferro-magnesian minerals, the less would be the proportions of free alumina and of free iron oxide. The figure shows that this is generally the case. Table I shows that the amount of free silica in the soil samples is very low in all cases.

#### SUMMARY

1. A comparative study has been made of the physico-chemical and mineralogical properties of the bottom layer samples of some red and lateritic

soil profiles. The determinations made in this connection are maximum water-holding capacities and percentages of clay, of free  $\text{Fe}_2\text{O}_3$ , free  $\text{Al}_2\text{O}_3$ , free  $\text{SiO}_2$ , and of ferro-magnesian minerals present in the soils.

2. A fair linear relationship was observed between the percentages of clay fraction of soil samples from the bottom layers of the profiles and their maximum water-holding capacities.

3. In general it has been found that the greater the proportion of ferro-magnesian minerals, the less are the percentages of free sesquioxides in the samples from the bottom layers.

4. The amount of free silica in the samples of the bottom layers is very low in all cases.

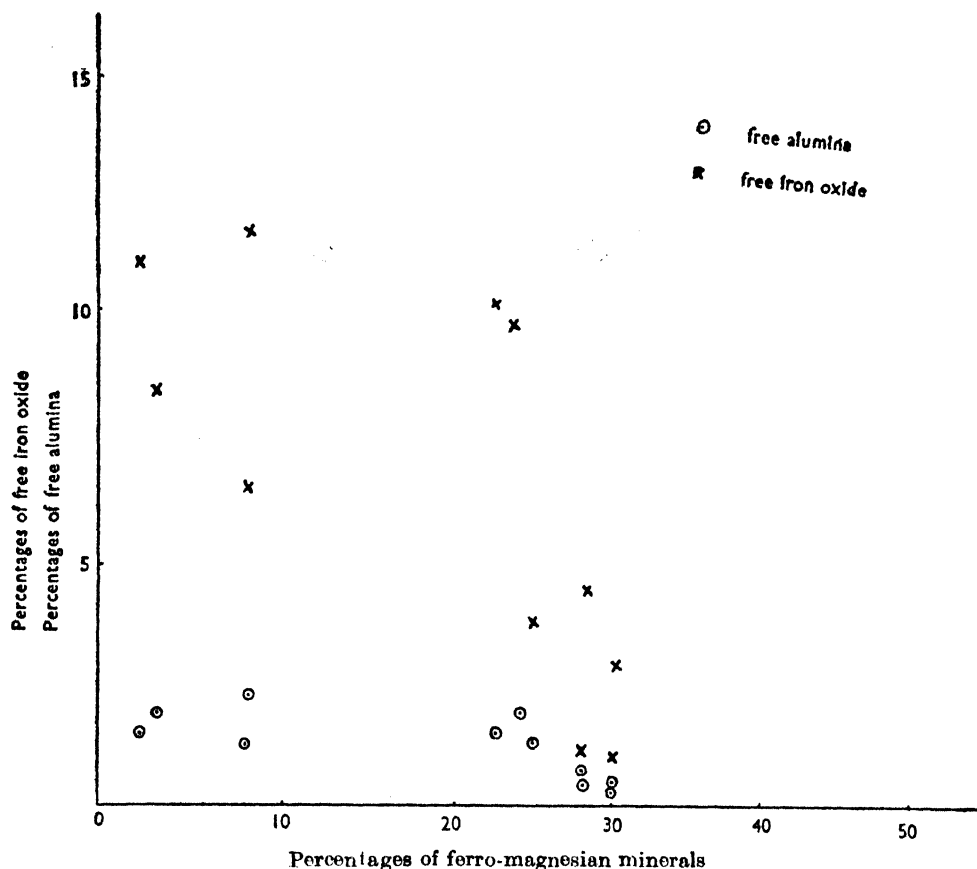


FIG. 2. Relationship between the percentages of free iron oxide and free alumina with ferro-magnesian minerals

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# UTILIZATION OF PRESS-MUD, CANE-TRASH AND BAGASSE IN THE CANE FIELDS

## I. COMPOSTING BY AEROBIC DECOMPOSITION

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**C**ONTINUOUS cropping of virgin land without periodical addition of any manure is certain to result in a rapid and continuous fall in the total organic matter, nitrogen and moisture-retaining capacity of the soil. Consequently, there will be a reduction in quality as well as quantity of successive crops. In order to safeguard against this, the ingredients taken out of the soil by any crop should be replaced so that the next crop may not suffer due to their absence. The proportion of the essential constituents of the soil can be maintained by the addition of synthetic fertilizers when the deficiency is in nitrogen, calcium, potash or phosphates, and of farmyard manure, green manure or oil cake if the soil is poor in organic matter also.

It is well known that humus or soil organic matter is responsible for soil-fertility in a number of ways such as improved tilth, water-retaining power etc. Its deficiency which cannot be rectified by the addition of artificial fertilizers can be made up only by the supply of organic matter in a readily assimilable form. The vegetable waste product added to the soil must contain sufficient combined nitrogen for rapid decomposition which takes place only in proportion to the available combined nitrogen present.

The deficiency in humus which most Indian soils suffer from, is generally made up by the addition of oil-cake, green manure etc. Since oil-cakes are expensive and not available in sufficient quantity in all parts of India, it is desirable to use cheaper humus-forming materials like straw and other cellulosic material. 'Sheet composting' has been suggested for utilizing these, but it would certainly be better if these materials are composted outside the field before being applied to the land, so that the manure will have preformed humus for immediate utilization by the crop. The ripe compost must be in the form of a finely divided powder, with a C : N ratio as close to 10 : 1 as possible.

As a consequence of the development of sugar industry in India, cane trash, filter press cake and molasses and in some factories bagasse also have become available in large quantities. In many cases, even their disposal—not to speak of their utilization—has become a problem to the sugar factory owners. Considerable work is already being done on utilizing molasses to the best advantage; we have therefore confined our attention to evolving the best method of using press cake, cane trash and bagasse on the land. The following table gives the quantities of cane crushed during the last five seasons in the United Provinces, Bihar and in the whole of India by sulphitation factories.

	United Provinces (in million tons)	Bihar (in million tons)	India (in million tons)
1935-36 . . . . .	5.2	2.14	8.8
1936-37 . . . . .	5.9	2.7	10.2
1937-38 . . . . .	5.4	1.9	8.8
1938-39 . . . . .	3.26	1.36	6.2
1939-40 . . . . .	7.0	3.5	13.1

Season 1938-39 was an unusually poor one and need not be taken into consideration. The average quantities of cane crushed per season will therefore be :

United Provinces	Bihar (in million tons)	India
5.9	2.56	10.2

While 20 per cent of cane forms trash, the quantity collected in sugar factories is usually only 1.1.5 per cent. The amount of filter press cake produced is found from the data available, to be about 2.5 per cent on the weight of cane crushed. The cake fresh from the presses contains about 60 per cent moisture and, therefore, the dry matter in the press cake produced may be taken to be 1 per cent on the weight of cane crushed. On this basis, the two products the disposal of which is a problem in Indian sugar factories will amount (in tons) per season to :—

	United Provinces	Bihar	India
Cane trash . . . . .	59,000	25,600	1,02,000
Filter press cake . . . . .	1,47,500	64,000	2,55,000
Dry matter in the cake . . . . .	59,000	25,600	1,02,000

These are enormous quantities of potentially useful materials and it is very desirable that attempts should be made to use them in the shape of manure for cane from which they are produced. It would be ideal if they could be used directly for this purpose. Sporadic attempts have been made in other countries to use cane trash directly on the fields and allow it to decompose there, but without much success. Filter press cake is being used as manure in a number of countries and in India also. In the United States of America, it is reported that press cake is almost exclusively used for manurial purposes, especially on the stubble canes on account of its phosphatic constituents. In most of the factories in the United States of America, it is stated, the cake as it comes out of the press is directly transported to the fields and applied to stubble canes ; in others, it is stored on the factory premises in a pit and transported later when dry.

In Mauritius, it is recorded that all the filter press cake produced, the average composition of which is given in Table I, is used on the fields as manure. It is almost exclusively used for virgin canes either at planting or when they are 5-6 months old. The ashes from the bagasse furnace are mixed with press cake or molasses or even with bagasse, giving a compost which has been called 'Saccharogene'. Bagasse which is in surplus has also been tried on the soil mixed with other substances, but it is stated that it remains in an unchanged state for a considerable time.

Filter press cake is a valuable manurial material containing potash, calcium, phosphate, nitrogen and organic matter. Unfortunately, many detailed analyses of Indian press-muds are not available, but analysis of a few samples has shown that they differ in composition from those of other countries. Table I gives a fair idea of the composition (on a dry basis) of press cake from Indian sulphitation factories, although there will be considerable variation between the products of different factories and between the products of sulphitation and of carbonatation factories, the latter containing far more mineral matter than the former. For purposes of comparison, average composition of the material in Hawaii and Mauritius is also given.

TABLE I

*Composition of press cake from Indian sulphitation factories*  
(Dry basis)

Constituent	Raval- gaon sugar factory	Experi- mental sugar factory	Mansur- pur factory	Another factory	Hawaii	Mauri- tius
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Organic matter	74.0	67.0	..	63.0	..	..
Nitrogen	1.47	1.0	1.43	1.0	1.85	1.14
P <sub>2</sub> O <sub>5</sub>	4.4	4.2	..	5.0	8.9	2.04
CaO	10.6	9.8	..	10.0	11.3	..
K <sub>2</sub> O	..	2.3	..	7.0	0.4	0.87

Press cake can be used for the benefit of the land either by itself or in admixture with other waste materials. It may also be applied after composting mixed with artificial fertilizers like ammonium sulphate or with fertilizer mixtures at present supplied to the ryots by some Departments of Agriculture. When used by itself directly it can be ploughed into the fields or allowed to decompose in a heap and then spread on the land. When used along with other agricultural waste materials, it is preferable to prepare composts to convert the organic matter in it and in the other materials to a form in which they can be readily assimilated by the crop. The easily available waste product which suggests itself is cane trash or in some cases, bagasse. Such green vegetable refuse as is available should also be used. In most of the factories, cane is supplied in bullock carts and cattle urine and cowdung are available and can be used as starters. In addition, diluted molasses and effluent sludge

can serve the same purpose, effluent water being used for preparing cowdung slurry. The use of press-mud mixed with other waste material is to utilize other waste products and also to obtain an increased yield of manure containing larger quantities of humus.

Different processes of composting have been developed and the methods have more or less been standardized ; for new materials, however, experiments have to be conducted to determine the period of composting, composition of the compost and the most suitable process. The well-known process is the Indore Process and various modifications suitable for application to different raw materials have been evolved. In the case of sugar factory products, whatever the process of composting adopted, either the raw materials or the finished products have to be stored for 4-6 months if they have to be applied to the land at the time of planting or a little earlier. For application to the ratoon crop, this long storage may not be necessary, as the programme of work can be so arranged as to have the compost ready just when it is required. It was, therefore, considered necessary to investigate thoroughly all the possible methods of using filter press cake, cane-trash and bagasse before recommending to the factory owners and cultivators the best method of utilizing these for the benefit of the land.

#### EXPERIMENTAL

##### *Composition of the materials used*

Filter press cake (sulphitation) contained moisture, 62.6 per cent. The cake after drying in the sun for 15 days in a thin layer on the ground contained 1.1 per cent moisture, 1.38 per cent nitrogen and 40 per cent carbon.

Air-dried cane trash contained moisture 7 per cent ; N, 0.24 per cent ; C, 28.0 per cent (on dry basis). Partially dried bagasse contained 30 per cent moisture, 0.14 per cent N and 40 per cent C (on dry basis).

Factory molasses contained 0.27 per cent N and 24.0 per cent C. Cowdung had 61.5 per cent moisture, the nitrogen and the carbon in the oven-dry sample being 0.92 per cent and 14.0 per cent respectively.

##### *Composting*

For the preparation of the heaps, sun-dried press-mud (sulphitation), cane-trash cut into lengths of 2-3 in. and small lengths of bagasse well-dried in the sun for 8-10 days were used. The requisite quantities of materials (the total weight of each heap was 8 cwt. the proportion of the ingredients varying in different cases, as given in Table II) were mixed together thoroughly with the activators and made into a heap of suitable dimensions. Temperature inside the heap was noted periodically to have an idea of the progress of decomposition. The maximum temperature attained in most heaps was about 70°C.

(A) The heaps were subjected to three turnings, once after 15 days, again after another 15 days and finally a month later, with the addition of a thin slurry of molasses and cowdung prepared with effluent water. Water was sprinkled occasionally if the heaps became too dry. Composts were taken to be ready when the heaps had developed a crumbled powdery structure and a grayish black appearance. Nitrogen and moisture were determined at this stage. Ten heaps (experiments 1-10) of different compositions were prepared in duplicate and composted by this method.

TABLE II

*Loss of nitrogen and dry matter from heaps of different composition*

Expt.	Composition of the heap						No. of turnings	Time of composting in months	Percent- age loss of dry matter	N per- centage in the compost (on dry basis)	Per cent loss of nitro- gen
	Pres- mud	Cane- trash	Bag- asse	Molasses and cowdung per cent of each on the wt. of heap	C : N ratio	N per cent					
1A XX	1	1	...	2 per cent	41 : 1	0.83	3	5	49.6	1.43	10.7
1B	1	1	...	"	41 : 1	0.83	3	6	53.7	1.42	19.0
2A	3	1	...	"	33 : 1	1.1	3	6	61.0	1.69	38.4
2B	3	1	...	"	33 : 1	1.1	3	6	60.0	1.35	50.0
3A	2	1	1	"	42 : 1	0.79	3	6.6	64.5	1.33	40.0
3B	2	1	1	"	42 : 1	0.79	3	6.6	63.2	1.30	39.0
4A	1	...	1	"	52 : 1	0.76	3	7	63.0	1.17	42.4
4B	1	...	1	"	52 : 1	0.76	3	7	61.4	1.35	30.4
5A	3	...	1	"	37 : 1	1.06	3+1	6.8	59.7	1.56	37.6
6B	3	...	1	"	37 : 1	1.06	3	6	62.6	1.60	40.0
6A X	1	1	...	"	20 : 1	1.66	3+1	6.3	67.0	1.83	62.0
6B X	1	1	...	"	20 : 1	1.66	3	6	63.1	1.88	56.4
7A X	3	1	...	"	23 : 1	1.66	3+1	4.5	39.4	1.75	35.4
7B X	3	1	...	"	23 : 1	1.66	3+1	4.6	44.9	1.86	37.5
8A X	2	1	1	"	22 : 1	1.64	3+1	4.8	50.7	1.83	45.5
8B X	2	1	1	"	22 : 1	1.64	3+1	4.8	46.1	1.77	42.2
9A X	1	...	1	"	23 : 1	1.63	3+1	4.7	50.0	1.71	47.3
9B X	1	...	1	"	23 : 1	1.63	3+1	4.7	51.2	1.58	52.7
10A X	3	...	1	"	23 : 1	1.66	3+1	4.7	54.4	1.88	46.0
10B X	3	...	1	"	23 : 1	1.66	3+1	4.9	55.2	1.88	47.0
11A	1	1	...	1 per cent	41 : 1	0.83	90 (daily)	5.5	62.2	1.32	28.1
11B	1	1	...	"	41 : 1	0.83	90 (daily)	5.5	62.0	1.34	26.5
12A	1	1	...	2 per cent	41 : 1	0.83	17 (weekly)	4.6	55.0	1.29	22.9
12B	1	1	...	"	41 : 1	0.83	17 (weekly)	4.5	45.7	1.12	19.3
13A	3	1	...	"	33 : 1	1.1	17 (weekly)	4.5	53.0	1.44	28.9
13B	3	1	...	"	33 : 1	1.1	17 (weekly)	4.5	53.2	1.46	28.4
14A	1	...	...	1 per cent	29 : 1	1.38	11 (weekly)	4	54.4	1.55	42.5
14B	1	...	...	"	29 : 1	1.38	11 (weekly)	4	50.0	1.41	42.8
15A	1	...	1	2 per cent	52 : 1	0.80	None	12	30.3	0.89	22.8
15B	1	...	1	"	52 : 1	0.80	Do.	12	31.7	0.91	22.8
16A	1	...	1	"	52 : 1	0.80	Do.	12	38.4	0.91	29.6
16B	1	...	1	"	52 : 1	0.80	Do.	12	40.7	0.93	31.4

X Ammonium sulphate added to the heaps to raise the percentage of nitrogen

XX P<sub>2</sub>O<sub>5</sub> per cent in the compost : 3.3K<sub>2</sub>O per cent in the compost : 0.86

(B) Experiment 11.—Heaps were subjected to daily turning for a period of three months. Cowdung and molasses were added at each turning.

(C) Experiments 12-14.—Turning was done every week with addition of cowdung and molasses.

(D) Experiment 15.—Press-mud (4 cwt) and bagasse (4 cwt) were laid out in alternate layers of about 2-3 in. thickness each and the heap was made up of three such layers. Each of the layers was well mixed with a slurry of cowdung and molasses before the next upper layer was placed on it. The layers were not mixed and no turning was given.

(E) Experiment 16.—Press-mud (4 cwt) and bagasse (4 cwt) were well mixed with a thin slurry of cowdung and molasses and a heap of suitable dimensions was prepared. No turning was given.

### RESULTS

Considerable heat was developed in every one of these heaps within a day or two of preparation, the temperature inside the heaps after a week or ten days rising to 60-65°C. in many cases. After a lapse of 3-4 weeks, fermentation slowed down and little heat was developed. Table II contains the results obtained so far. The data obtained show clearly that valuable manure can be prepared from what are considered at present as 'waste products'.

Attempts were also made to use press-mud of carbonatation factories in the preparation of composts. These did not prove satisfactory because this press-mud contains a large proportion of inorganic matter. It contains only 7 per cent C and 0.6 per cent N. The addition of organic waste to balance the inorganic matter would reduce the percentage of nitrogen, which is already low, still further.

For adoption by the factories, the method of composting recommended has to be inexpensive. Our experiments have only this object in view. Method (B) is obviously unsuitable as labour required will be very large. The most promising methods for adoption on a large scale are (D) and (E). They involve little expense and the longer period of composting is not a disadvantage, as there is a good margin between the close of one crushing season and the next planting season.

It is observed from Table II that in all these methods, there is a loss of nitrogen varying from 20-50 per cent and of dry matter, 40-60 per cent depending on the composition of the heap. Excellent results have been claimed for hot fermentation method of composting by Acharya *et al.* [1939]. In this method, the heaps are subjected to both aerobic and anerobic fermentation, the former only for a short period in the beginning. Composting of the sugar factory waste products by this method is being examined, so that the losses may be reduced if possible.

### ACKNOWLEDGEMENT

Our thanks are due to Messrs G. N. Gupta and C. Parthasarathy for some analytical work given in this paper.

### REFERENCE

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# THE DISPOSAL OF POONA SEWAGE FOR IRRIGATION AND CROPPING

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(With Plates V and VI and two text-figures)

ONE of the most important questions in India is maintaining the fertility of the soil, specially in intensively cultivated areas. Every intelligent cultivator utilizes all his farm wastes by returning as much of it as possible to the soil to secure good results but the dearth of bulky manure is always keenly felt in intensively irrigated areas. Villagers round about the towns use city refuse and night-soil; green manuring is also resorted to when irrigation facilities are available. In large towns, the removal of excreta by baskets and carts is practised for economic reasons and is being gradually replaced by drainage system for sanitary reasons wherever possible.

From the analysis of effluent received from the Poona city sewage, it is found that the nitrogen contained in it is roughly equal to 3 lb. per annum per head of population. The money value of these 3 lb. of N is equal to Rs. 1-8 on the basis of market rates and of N contents in oil-cakes, ammonium sulphate and other manures. If such valuable N is not utilized for irrigation but wasted elsewhere, the loss to national wealth due to such waste would amount to Rs. 3 lakhs per annum for Poona alone.

## METHOD OF DISPOSAL OF CITY SEWAGE

Sewage is at present being directly let into sea or large perennially flowing rivers. Where such facilities are not available, it has to be passed through septic tanks for anaerobic treatment and further through rotary filters for aerobic treatment. Recently the sludge portion of the sewage water is utilized for preparation of gas for power purposes. Sometimes effluent is required to be chlorinated or treated with flocculents. It is only then that it can be let into a *nalla* without any danger to public health. There is another way of utilizing the sewage, and this is by irrigation to crops. This latter method is described in the paragraphs to follow.

## POONA SEWAGE DRAINAGE AND IRRIGATION SCHEME

This scheme comprises (a) drainage from the Poona city, cantonment, suburban municipality, etc. The present volume of sewage received per head per day is about 35 gallons. (b) All this raw sewage is thus collected by gravity to a central pumping station at Bahiroba where this passes through screen and grit chambers for removal of *katchra* and sand. About 1,000 tons of sand are recovered annually. The screened sewage passes then through the balancing tanks; each tank is 250 ft. long and 36 ft. wide. The capacity of each of the tank compartments is about 0.6 million gallons making a total of 1.8 million gallons, for the three tanks. Sewage settles in these tanks for about four hours before it is pumped to the head of distributary 5 for

irrigation. (c) Sewage is further led on to a sump pit and pumped. The quantity pumped is about 7 million gallons per day at present. The static lift to which sewage is pumped is 88 ft. from suction level to delivery end.

The pumping set consists of four units, one electric unit and three oil units. The three oil units consist of Diesel engines, two cycle vertical type of 150 B. H. P. running at 300 revolutions per minute.

Sewage which is pumped passes through a rising main of 30 in. diameter and discharges at a distance of  $3\frac{1}{2}$  miles at distributary 5 of Mutha Right Bank Canal. There is also a branch pipe connected from the rising main which discharges at distributary 3. This is described in details by Inglis, Collett and Joglekar [1938].

The discharges are received in the masonry chambers at the distributary ends and are either diluted, if required, with canal water which is close by or is allowed to pass in the raw form for irrigation purposes. Fig. 1 explains the lay-out clearly.

#### EFFLUENT IRRIGATION AREA

The area which receives sewage irrigation is called effluent zone which consists of areas under distributaries 3, 4, 5 and 6 of Mutha R. B. Canal (Fig. 1). The total area suitable for sugarcane cultivation is about 3,000 acres, but

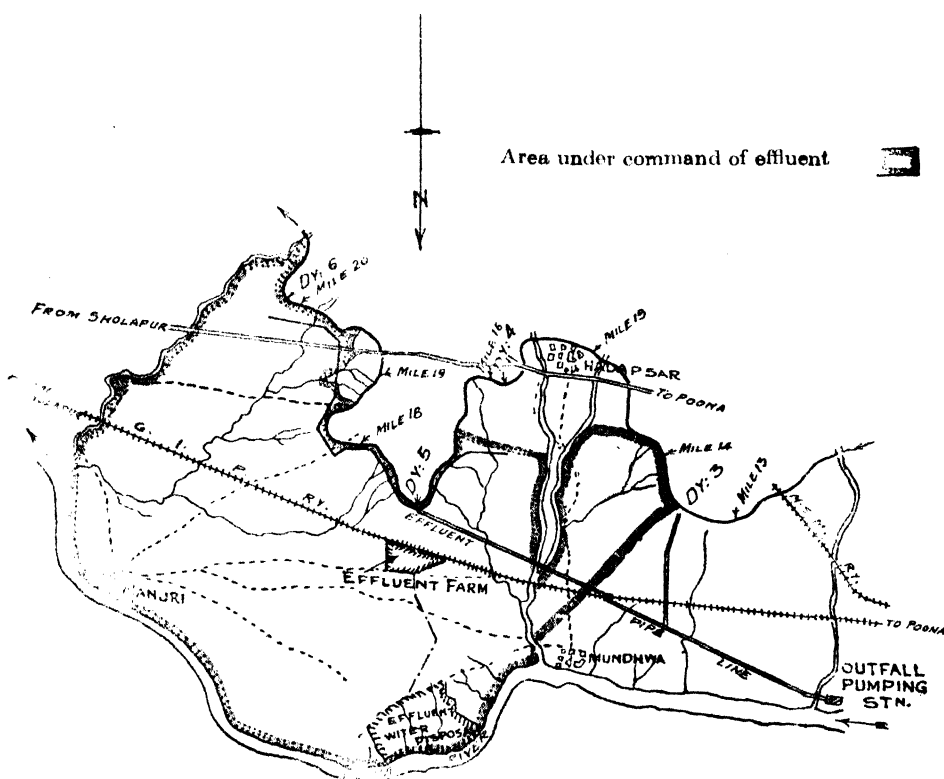


FIG. 1. Index plan showing effluent zone, Mutha Right Bank Canal, from mile No. 13 to 20 (scale 1 in. =  $3\frac{1}{2}$  mile)

only 800—1,000 acres at the most are under sugarcane at present, while the sewage received from Poona is sufficient for supplying N requirements to about 2,000 acres on the basis of 300 lb. of N which has been found to be adequate for producing a good crop of sugarcane. As only half the area is therefore normally under sugarcane, Government have acquired an area for disposing of surplus effluent when it is not required for sugarcane. This is done during the latter half of *rabi* season as it is beneficial to cut off sewage at least two months prior to harvesting of sugarcane.

#### ANNUAL DISCHARGES OF SEWAGE AND ITS MANURIAL VALUE

Table I gives the discharge of sewage received during the preceding five years.

TABLE I

*Discharge of sewage, total nitrogen and area irrigated in the zone*

Year	Discharge of sewage in c.ft. per second	Discharge utilized in cusecs	Discharge surplussed in winter disposal area cusecs	Total N parts per 100,000	Area sufficient for cane on 300 lb. N basis	Actual perennial area irrigated	Remarks
1935-36 . . .	10.23	9.82	0.41	3.53	Acres 1880	Acres 999—30	One cusec one hour—22,500 gallons
1936-37 . . .	9.91	9.55	0.36	3.55	1787	1270—40	
1937-38 . . .	8.55	8.15	0.40	3.79	1620	367—20	
1938-39 . . .	10.43	9.46	0.97	3.50	1830	888—40	
1939-40 . . .	10.77	10.77	...	3.31	1782	1005—20	
Average . . .	...	...	...	...	1780	906—0	

There is a gradual fall in the total N contents from 1937-38 which is due to increased usage of water in the city. The current year shows highest discharge and is naturally accompanied by an appreciable decrease in the total N contents. The average of five years figures shows that the area for which N sewage was sufficient at 300 lb. per acre was about 1,780 acres, whereas an area of 906 acres only has been irrigated.

The average of weekly results of free ammonia, albuminoid ammonia, suspended solids and other useful ingredients during the year 1939-40 are given in Table II.

TABLE II

*Manurial ingredients in Poona sewage during 1939-40*

(Parts per 100,000)

Season	Free and saline ammonia	Albuminoid ammonia	Total N	Potash as K <sub>2</sub> O	Phosphoric acid as P <sub>2</sub> O <sub>5</sub>	Calcium as calcium oxide	Suspended solids
Hot Weather . .	2.51	0.92	3.43	0.521	1.35	14.287	44.10
Monsoon . . .	2.36	0.79	3.15	0.377	1.737	13.844	44.10
Rabi . . .	2.41	0.84	3.25	0.396	1.969	14.008	53.30

The average discharge was 10.77 cusecs while the suspended solids on an average were about 47 parts per 100,000. This would work out to 5,040 tons or 10,000 carts. Assuming that half of this settles in the water channels, it can be safely said that each acre of the 1,000 acres of cane irrigated in all gets on an average 5 cart-loads of this matter in addition to the nitrogen contained in sewage. It will be seen from Table II that the Poona sewage as well contains an appreciable amount of potash and phosphoric acid and hence is a complete manure. These figures, however, represent the total potash and phosphoric acid and not necessarily the quantity available. The latter is under further investigation.

It may be noted in this connection that the experiments at the Padegaon Research Station proved that the addition of  $P_2O_5$  to the extent of 100 lb. in addition to the usual standard dose of N top dressing, with sunn-hemp before cane, gave results which increased the tonnage by 19 per cent accompanied by high *gul* to cane recovery.

At present sewage being in excess of the requirements, each acre of cane receives about 600 lb. N or nearly double the quantity required. No evil effects of these heavy doses on the quality of *gul* have, however, been noticed so far.

It will be seen that the effluent zone of Poona is a big estate growing about 1,000 acres of sugarcane and other two seasonal garden crops and perennial grasses, vegetables and fruits. These are freely sold in the market with the usual precautions of washing in case of the former and no harmful results have been observed. Sugarcane grown under sewage irrigation is used in Poona and Bombay, both for chewing and for sugarcane juice as a sweet drink.

As N in sewage is in liquid and easily assimilable form and the total scheduled N is applied in many doses, crops are more benefited than was when manure, either farmyard manure or concentrated manure, was applied in the normal way. In the latter case some of the manure is unutilized which is evident from the residual effect of manure on succeeding crops.

Crops irrigated with sewage exhibit a deeper green colour than those irrigated with canal water.

#### EXPERIMENTS AT EFFLUENT FARMS \*

Sugarcane is a principal crop on this side. Several experiments with this crop were tried at the Effluent Farm, Hadapsar, in medium black soil 12-18 in. deep overlying murrum and described by Inglis [1927]. Dr Basu surveyed the soil of this area and has classified this soil as G type† according to the genetic soil classification described in details by Basu and Sirur [1938].

The rotation followed is biennial, i.e. cane and *dhaincha* (*Sesbania aculeata*), a green-manure crop, are grown alternately. Sugarcane receives sewage

\*Up to 1929 the sewage received from Poona was treated and the effluent obtained therefrom was utilized for irrigation at Hadapsar and hence the Farm is known as Effluent Farm. Since 1930 the sewage is passed directly on to the land. However, the title remains unchanged and the farm is still known as the Effluent Farm.

† Brown-coloured soil 15-24 in. deep over lying murrum (decomposed trap) consisting of about 50 per cent clay and 10-15 per cent silt, pH values near about 8.0, containing moderate quantities of humus and nitrogen. There is low CaO/MgO ratio indicating a general inferior drainage condition of the soil.

throughout, while *dhaincha* requires only one irrigation. Thus out of 24 months, the land receives sewage irrigation for 16 months.

#### VARIETIES UNDER SEWAGE IRRIGATION

Seven important varieties of sugarcane including *Pundia* were tried. They were all planted in January. In one series, normal dose of effluent (300 lb.) supplemented by canal water was given, while in the other sewage irrigation was continued till harvest. The experiment was in replicates. Harvesting was done by February next year, after 13½ months. The out-turns are given in Table III.

TABLE III  
*Performance of different varieties*

Name of variety	Normal dose (300 lb. N)				Sewage irrigation throughout (roughly 900 lb. N)			
	Out-turn of cane in tons	Out-turn of <i>gul</i> in tons	Brix	Purity (per cent)	Out-turn of cane in tons	Out-turn of <i>gul</i> in tons	Brix	Purity (per cent)
<i>Pundia</i>	37.85	4.37	18.6	81.5	36.31	3.94	17.9	76.7
Co 417	50.74	6.06	19.0	81.6	50.96	5.66	18.7	79.6
Co 408	53.91	6.17	20.3	80.1	47.12	5.34	20.3	81.3
Co 419	49.87	5.95	21.2	83.7	52.25	6.44	21.4	85.1
POJ 2878	47.35	5.14	19.2	82.0	45.80	5.39	20.4	88.3
Co 411	49.87	5.07	18.9	81.1	47.28	5.27	18.8	81.4
POJ 2883	40.17	4.98	20.8	86.9	41.74	4.95	20.9	87.8

The results of *gul* weights show that Co 419 and POJ 2878 are benefited by continued effluent. Co 411 also shows a small increase. There is a depressing effect of continuing effluent till harvest in the case of Co 417, Co 408, POJ 2883 and *Pundia* (local cane). Further experiments with different suitable varieties are in progress. Plate V, fig. 1 shows the growth of important varieties.

Out of the above, POJ 2878 and Co 419 were further tested under heavy dose experiments in the following year on plots Nos. 111—116 of the Effluent Farm. These varieties were planted in December and harvested after 12-14 months on maturity, which was noted by taking brix readings at intervals. The average out-turns from four replicates are given in Table IV.

TABLE IV  
*Comparative out-turns of Co 419 and POJ 2878 varieties under heavy doses of N*

trial no.	Description of experiment	Name of variety	Cane wt in tons per acre	<i>Gul</i> wt in tons per acre	Brix	Per cent of <i>gul</i> to cane	Remarks
A	300 lb. of N, i.e. normal dose	POJ 2878	51.44	5.79	18.35	11.25	
		Co 419	57.92	6.62	20.31	11.40	
B	*900 lb. of N, i.e. crop raised on sewage irrigation alone	POJ 2878	52.66	5.91	18.31	11.23	
		Co 419	60.60	6.86	19.23	11.32	

\* The experiments on seeing the effect of sewage irrigation alone on crop and soil are mainly due to Mr U. N. Mahida, B.E., I.S.E., now Deputy Secretary to the Government of Bombay, P. W. D. His personal observations on this aspect of the problem have greatly stimulated research of much practical importance.



FIG. 1. Growth of important varieties under trial at the Effluent Farm and effluent zone ( 1. Co 419 heavy dose ; 2. Co 419 normal dose ; 3. EK 28 normal dose of sewage ( from cultivator's fields ) ; 4. POJ 2878 ; 5. Co 426 ; 6. POJ 2883 ; 7. Pundia ( Local cane ) )



FIG. 2. Growth under heavy doses of nitrogen : A. 300 lb. of nitrogen and sewage diluted with canal water ; B. 900 lb. of nitrogen with sewage irrigation alone ( Date of planting 7-1-1939, variety Co 419, age 11 months )

[ Note the loose tilth of the ploughed lands lower down which have been under sewage irrigation for 20 years ]



FIG. 1. Cultural treatment,  
Co 419 variety, 12½  
months old

Left : Earthing up

Right : No earthing up

(Note the poor growth under  
'no earthing up')

FIG. 2. Cultural treatment  
Co 419 variety 12½  
months old ( inside  
view of sub. plots )

Left : Earthing up

Right : No earthing up

(Note the lodging of 'no-  
earthing up')



FIG. 3. Cultural treatment  
Co 426 variety 12½  
months old

Left : Earthing up

Right : No earthing up

(Note the good growth of  
treated cane on the  
left)

In item A, cane was given 300 lb. of nitrogen in 10 months and was further supplemented by canal water, while in item B sewage irrigation alone was given throughout. Plate V, fig. 2 shows the growth of cane under normal and continuous sewage irrigation. There is a distinct difference in the growth of cane between the two treatments. Treatment B, with a large dose of N and with sewage irrigation alone, gives more vegetative growth than treatment A. The difference in yield between A and B is, however, not appreciable. But the experiment shows that it is possible to raise a sugarcane crop under sewage irrigation alone with proper treatment, as detailed further.

#### EFFECT OF SEWAGE IRRIGATION ON A RATOON CROP

POJ 2878 sugarcane variety was tried with varying doses of N from 300 to 600 lb. of nitrogen. This was planted as usual in the month of December 1937 and harvested in January 1939. Ratoon was kept during the year 1939 and harvested in 1940. The varying doses of N in the form of effluent irrigation were continued to the ratoon cane also. The results are shown in Table V.

TABLE V

*Out-turns of POJ 2878 under ratoon condition with varying doses of nitrogen compared to the out-turns of plant cane*

Dose of N in lb. per acre	Out-turn of cane in tons per acre (plant cane)	Out-turn of cane in tons per acre (ratoon)	Out-turn of <i>gul</i> in tons per acre (plant cane)	Out-turn of <i>gul</i> in tons per acre (ratoon)	Recovery of <i>gul</i> to cane (plant cane) per cent	Recovery of <i>gul</i> to cane (ratoon) per cent
300 . .	45.42	35.52	5.35	4.33	11.78	12.15
400 . .	45.50	37.3	5.45	4.40	11.99	11.89
500 . .	45.36	39.56	5.37	4.86	11.60	12.30
600 . .	46.97	39.42	5.33	4.57	11.34	12.02
Only sewage irri- gation roughly 700 lb. N	47.58	42.51	5.89	4.70	12.12	11.54

The results show increased yield of *gul* with increased doses both in the case of plant cane and ratoon cane except 500 lb. dose in the case of ratoon and 600 lb. dose in the case of plant cane.

It must be noted that except sewage irrigation no other manure was given to ratoon crop. Great care was taken in doing timely partial and complete earthing up operations. The results of ratoon tried with Pundia (local cane) Co 417 and Co 419 are given in Table VI,

TABLE VI

*Out-turns of local cane and Co varieties under plant and ratoon condition*  
(Per acre)

Variety	Description	Ratoon		Plant cane	
		Out-turn of cane in tons	Out-turn of <i>gur</i> in tons	Out-turn of cane in tons	Tons of <i>gur</i> per acre
Pundia	Normal dose 300 lb.	30.60	3.32	..	4.42
Co 417	Do.	40.41	4.64	47.24	4.79
Co 419	Do.	35.03	4.39	48.42	5.13

EFFECT OF SEWAGE IRRIGATION ON *ADSALI* CANE

In this zone, the problem of sewage disposal is of great importance, especially in the months of November—February when plant cane normally does not require sewage. Some of the surplus sewage could be utilized by *adsali* cane planted during monsoon. This experiment was continued with a view to finding out which of the new cane varieties gave best results under this condition. In previous experiments Pundia had failed to grow as an *adsali* crop. Hence four important varieties were tried. Planting was done on 15 June and 1 August and harvesting was done after 18 and 16 months respectively when the crop showed signs of maturity. The out-turn was as shown in Table VII.

TABLE VII

*Out-turn of varieties under adsali plantation*  
(Per acre)

Name of variety	June planting			August planting			June plantation difference from the control	August plantation difference from the control
	Cane weight in tons	No. of canes at harvest	Brix	Cane weight in tons	No. of canes at harvest	Brix		
POJ 2878	70.1	42,225	18.28	42.6	35,131	17.37	2.8	5.6
POJ 2883	67.3	35,400	17.89	37.0	31,781	19.29	Nil	Nil
Co 419	76.8	54,450	15.18	48.9	44,906	17.44	9.5	11.90
Co 411	86.9	48,750	16.11	49.1	36,469	16.23	19.6	12.10
Note.—Significance for both the June and August plantation combined				Significance figures			7.92	5.77

The experiment was replicated four times. The statistical treatments of the results showed June plantation to be significantly better than August plantation, while considering the two plantations separately Co 419 and Co 411 gave significantly higher yields than the rest.

It will be seen that Co 411 gave the highest yield of cane, about 87 tons in the case of June plantation. There was also profuse tillering and highest

number of canes were recorded in the case of Co 419 variety. These results show that Co varieties are more suited for planting in June and August than POJ varieties. Of the two, plantation in June is much superior to August plantation from the point of view of growth.

#### *Early cane plantation*

Sugarcane varieties were also planted in October. It was found that POJ 2878 and Co 419 gave respectively 3 and 4 tons of cane more than January planted cane under similar soil conditions.

#### CULTURAL TREATMENT UNDER SEWAGE IRRIGATION

Under the conditions prevailing in the effluent zone, the necessity of timely cultural operations cannot be over-emphasized. The most important of these operations are the partial earthing up and earthing up. The first operation removes unnecessary rootlets (*jarwa*), loosens the top soil and secures better ventilation. It also brings some fresh soil from the ridge nearer the root zone. Irrigation is given five to six days after this operation. Good effects are seen a month after this operation. The next operation of earthing up is done when the canes show two or three well-developed nodes. During this operation the soil is loosened by pickaxes which cuts off the unnecessary dead roots and rootlets while the loosened fresh soil from the ridge is shifted to the cane rows in the furrows. Thus, after this operation, the cane is on the ridge. This operation is started two days after the sewage irrigation is given which is followed by a light sewage irrigation (called *bore padne*) after four days. This latter irrigation can be omitted if there are rains. This operation greatly stimulates the crop later. It is shown by Regc and Wagle [1939] that this operation can be omitted for a sugarcane crop when the tonnage is about 40 or so. To test if this operation can as well be recommended for omission by sugarcane growers in the effluent zone, a systematic experiment was laid out with important varieties and the results are shown in Table VIII.

TABLE VIII

*Out-turn of Co 419 and Co 426 cane varieties under cultural treatment*

Treatment	Co 419 variety cane yield		Co 426 variety cane yield		Co 419 differ- ence from the control	Co 426 differ- ence from the control
	In tons	Brix	In tons	Brix		
1. Partial earthing up and earthing up	47.57	21.12	42.70	18.82	12.59	10.9
2. No partial earthing up but only earthing up	47.03	20.63	44.50	18.83	12.05	12.6
3. Partial earthing up but no earthing up	36.27	19.78	34.90	16.79	1.29	3.0
4. No partial earthing up. No earthing up	34.98	20.25	31.90	17.87	Nil	Nil
Significance figures Co 419	..	..	..	..	7.71	..
Significance figures Co 426	..	..	..	..	..	9.09

These results show the great influence of earthing up on yield and also on brix. Plate VI, fig. 1 (front view of the experiment) shows the difference of growth between earthing up and non-earthing up, fig. 2 shows the heavy lodging caused due to non-earthing up even for a 40-ton crop and fig. 3 is for Co 426 variety under similar treatments. These results clearly show that under the conditions prevailing in this zone this important operation has to be done regularly to ensure a good crop. This system is closely followed by the irrigators on the Mutha Canals, specially of this zone, under the supervision of the staff of the Effluent Farm.

### FODDER CROPS

Regular experiments were laid out in the same type of soil (G type) to see the out-turns of different fodder crops under sewage irrigation.

The out-turns are given in Table IX.

TABLE IX  
*Out-turn of different fodder crops per acre*

Name of crop	Canal irrigation under standard manure dose (lb.)	Sewage irrigation only (lb.)	Remarks
<i>Hundi jowar</i> * . . .	12,480	33,561	Hot weather fodder
<i>Nilwa jowar</i> * . . .	27,736	Nil	Monsoon fodder
Maize . . . . .	15,000	33,000	
Berseem . . . . .	29,520	31,160	Considering six cuttings
Lucerne . . . . .	26,040	54,347	For one year

\* *Andropogon sorghum*

The out-turn under sewage irrigation is more than double the out-turn under canal irrigation.

### EFFECT OF SEWAGE IRRIGATION ON SOIL

The object was to see if any deterioration in soil tilth was noticeable under continuous sewage irrigation. For this purpose, careful selection was made of soils in the effluent zone, irrigated by canal water and under continuous sewage irrigation for 20 years. Fig. 2 shows the exact position of profiles examined. In all five comparisons were made. The results are given in Table X.

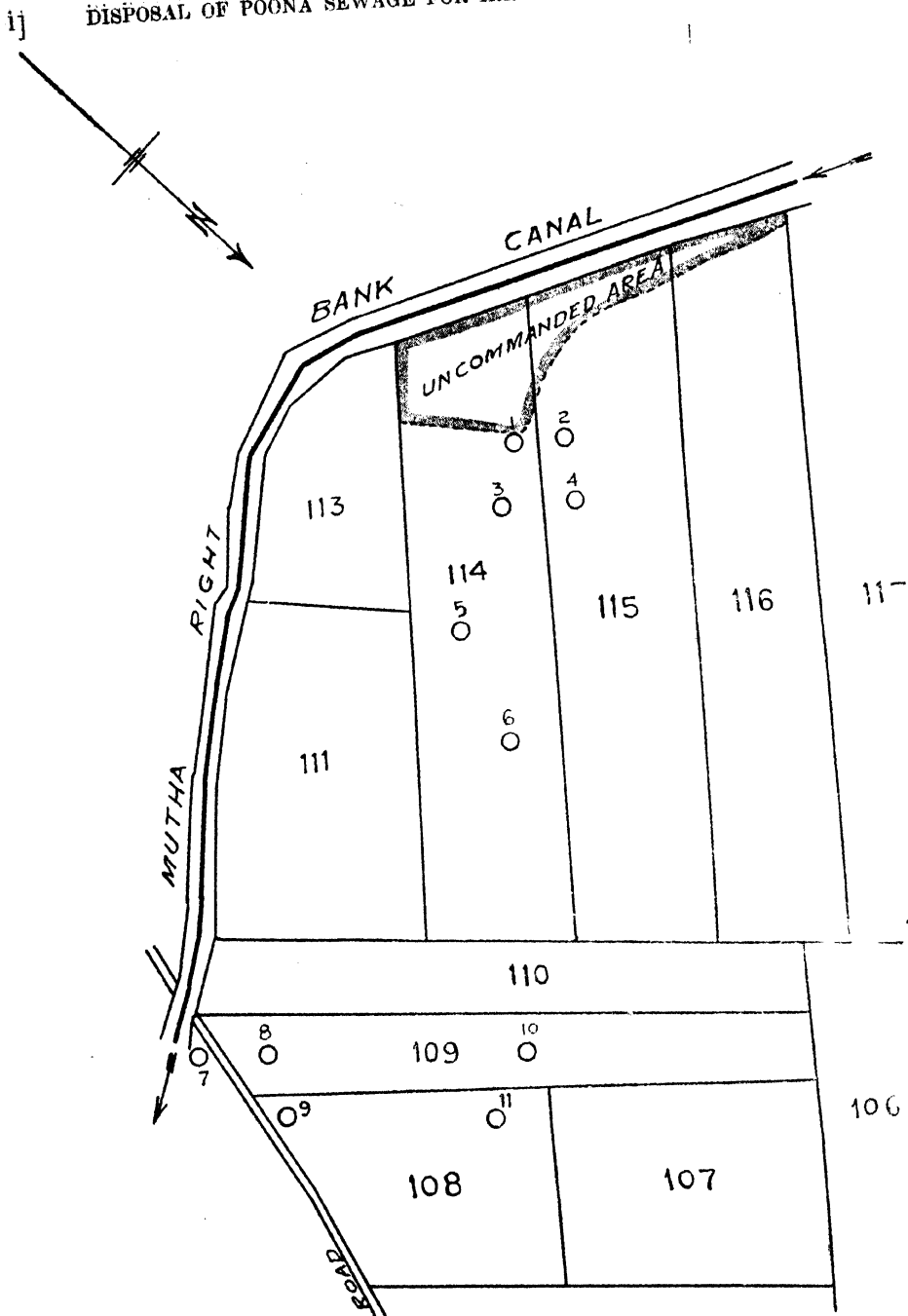


FIG. 2. Plan showing positions of profiles where soil samples were taken for comparative study of soil under and outside sewage irrigation (scale 1 in. = 660 ft.) Profiles Nos. 1, 3, 5, 8 and 10 are under canal irrigation and Nos. 2, 4, 6, 9, 11 and under continuous sewage irrigation; profile No. 7 under dry crops only

TABLE X

Results of soil tests under sewage irrigation and canal irrigation alone

Profile No.	Soil depth (in.)	Canal irrigation					Sewage irrigation				
		Capillary rise in 300 minutes (in.)		pH values in		Per cent $\text{CaCO}_3$	Per cent humus	Per cent soluble salts	Capillary rise in 300 minutes (in.)		pH values in
		In water	In $\text{N NaCl}$ solution	Distilled water	$\text{N KCl}$ solution				In water	In $\text{N NaCl}$ solution	Distilled water
I . . . . .	0-6	2.55	3.25	8.24	6.71	2.8	0.597	0.075	2.55	2.40	7.71
	6-12	1.85	2.20	7.98	6.94	2.75	0.944	0.075	1.60	2.15	7.94
	0-6	2.10	2.50	7.52	7.18	3.20	1.68	0.10	1.60	1.55	7.84
	6-12	1.55	1.40	7.58	7.18	3.20	1.82	0.125	1.55	1.55	8.32
II . . . . .	12-24	1.05	1.35	8.00	6.66	3.25	...	0.15	1.30	1.25	8.32
	0-6	1.20	1.15	7.51	6.24	2.90	0.669	0.075	2.15	2.05	7.48
	6-12	1.10	1.22	7.54	6.34	2.82	0.84	0.125	2.50	1.80	7.58
	0-6	1.30	1.65	7.82	7.07	3.35	0.98	0.15	1.45	1.40	7.84
IV . . . . .	6-12	1.20	1.45	7.82	6.82	3.10	0.96	0.10	1.15	1.05	7.57
	12-24	1.20	1.60	7.92	7.42	4.67	0.79	0.15	2.15	2.10	7.32
	0-6	1.70	1.75	8.24	7.44	4.75	1.46	0.17	1.00	1.05	7.32
	6-12	2.95	3.90	8.24	7.30	3.87	0.62	0.16	6.0	6.0	7.81
V . . . . .	0-6	1.15	1.80	8.24	7.25	2.85	1.03	0.10	Profile never under any irrigation but under monsoon cropping		
	6-12	1.35	1.65	8.06	7.14	2.75	0.72	0.10			
	0-6	1.15	1.80	8.24	7.25	2.85	1.03	0.10			
	6-12	1.35	1.65	8.06	7.14	2.75	0.72	0.10			

NOTE.—Humus was estimated by Sigmund's method modified in this Laboratory. Soluble salts were estimated by Dionic water tester

The capillary rise tests in canal water and in *N* NaCl solution for profiles under canal and sewage irrigation show decidedly less difference between the two readings in the case of effluent irrigation. In certain cases, the capillary rise with *N* NaCl is slightly less than with ordinary water which show considerable soil improvement under sewage irrigation. The *pH* values were found out by antimony electrode [Puri, 1932] in distilled water and in *N* KCl solution and gave similar indications. The *pH* values are decidedly low in the case of sewage irrigated profiles, while the difference between distilled water value and *N* KCl is small as compared to canal water ; this shows comparative deterioration under canal irrigation. The per cent calcium carbonate throughout the profile vary from 2.5 to about 4.5 with slightly more calcium carbonate under sewage-irrigated profiles. The humus contents are also slightly more under sewage-irrigated soils as compared to canal-irrigated soils under similar conditions. The total soluble salts show a little increase under sewage irrigation as compared to canal-irrigated soils but this quantity is as good as we find in normal soils of the type.

The exchangeable bases were found out by method advocated by Puri [1935, 1, 2]. Replaceable calcium was also found out by improved acetate method as advocated by him. The results are given in Table XI.

TABLE XI

*Exchangeable bases under canal irrigation and sewage irrigation (m. e. per cent)*

Profile No.	Soil depth (in.)	Under canal irrigation				Under sewage irrigation			
		Replace-able Na plus K	Replace-able Mg	Replace-able calcium	Total replace-able bases	Replace-able Na plus K	Replace-able Mg	Replace-able calcium	Total replace-able bases
I {	0—6	0.53	6.66	38.5	45.69	0.44	5.32	39.0	44.76
	6—12	0.53	7.82	39.5	47.85	0.35	6.18	43.30	49.84
II {	0—6	0.89	6.86	41.0	48.75	0.53	5.52	46.30	52.35
	6—12	0.80	7.24	40.0	48.04	0.36	5.32	46.50	52.18
	12—24	0.98	6.66	40.5	48.14	0.44	4.94	45.50	50.88
III {	0—6	1.068	8.48	37.0	46.55	0.56	9.24	37.0	46.804
	6—12	0.89	8.96	38.5	48.35	0.66	8.00	37.5	46.16
IV {	0—6	1.07	9.16	39.0	49.23	0.66	7.62	42.50	50.78
	6—12	0.98	8.58	39.0	48.56	0.94	9.60	43.50	54.04
	12—24	0.89	8.88	37.0	46.77	1.028	10.28	45.0	56.30
V {	0—6	1.07	5.70	42.0	48.77	1.50	7.80	42.50	51.81
	6—12	0.62	4.94	37.5	43.06	0.66	6.40	39.50	46.56
VI {	0—6	1.51	8.30	33.0	42.81	} Never under any irrigation but under dry crops			
	6—12	1.33	5.44	32.5	39.27				

These results show that out of 12 cases, 10 show a gain in exchangeable bases in the case of sewage-irrigated soils. It also shows an increase in replaceable

calcium. The percentage of monovalent bases are very low in both, and specially under sewage irrigated soils. These results indicate that calcium from sewage water (Table II) takes part in exchange phenomena and more calcium is made available under sewage irrigation. This point is under further investigation. However the results given do show that the soils in the effluent zone have not deteriorated by sewage irrigation as is commonly believed.

### Miscellaneous

In this zone, as the original potable water supply from wells, etc. were in danger of contamination due to the effluent irrigation, adequate piped water supply arrangements have been made for drinking purposes from specially constructed reservoirs for the purpose.

### SUMMARY

(1) A detailed description of the disposal of Poona sewage from the city to the pumping station, some three miles away from Poona, and thence to the cultivators' fields is given.

(2) The composition of Poona sewage is given, showing that it is a valuable and complete manure.

(3) Out-turns of sugarcane varieties, specially under sewage irrigation are given which show that high yields are obtained up to 60 tons of cane per acre in the case of Co 419 variety and up to 50 tons per acre in the case of POJ 2878. The latter (POJ 2878) is a better cane because of early maturity and good quality of *gul*.

(4) (a) Co 411 and Co 419 are very good as plant and *adsali* canes. As *adsali* plantation, cultivators prefer to grow also POJ 2878 and POJ 2883 as a mixture with Coimbatore varieties as it is observed that mixed crushing gives better quality of *gul* than with Coimbatore varieties only.

(b) POJ 2878, Co 417 and Co 419 ratoons well under sewage irrigation.

(5) Necessity of proper cultural operations under sewage irrigation is emphasized. Earthing up and partial earthing up operations are essential to maintain suitable soil conditions and get better returns.

(6) The out-turns from cane varieties and other fodder crops under sewage irrigation compare very favourably with the out-turns obtained elsewhere for similar crops under canal irrigation with bulky manures and artificial fertilizers [Vagholkar and Patwardhan, 1940] on sugarcane varietal trial, and of fodder crops as given in *Bulletin* No. 100 of Department of Agriculture, Bombay.

(7) Soils of the type studied do not show any deterioration under continuous sewage irrigation, provided suitable soil conditions are maintained; on the other hand some improvement is noticeable which is shown by capillary rise, pH values and exchangeable bases.

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# FIXATION OF ATMOSPHERIC NITROGEN IN LIVING FORMS

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## INTRODUCTION

THE most striking result of the researches of Pasteur in the last century is perhaps the revelation of a world of microbes incessantly at work taking part in all vital processes in relation to the maintenance of life on this planet. Among the great variety of micro-organisms that are responsible for the important processes in the soil, the nitrogen-fixing organisms have formed a subject of fascinating study.

In the middle of the nineteenth century Boussingault suggested that vegetable earth contains certain living organisms some of which take part in the fixation of nitrogen in the soil. Jodin [1862] first demonstrated that mycoderms growing in nitrogen free medium fixed nitrogen from the atmosphere. A few years later, Berthelot [1885] announced that he obtained increases in the nitrogen content of normal but not sterilized soils. He further showed that the rise in the organic nitrogen content of soils left uncultivated for a period of several months is due to the microbial activity. Still later, Hellreigel and Wilfarth [1888] discovered the nodule-organisms, *Rhizobia*, in the roots of leguminous plants and showed that these organisms, in association with the leguminous plants, bring about nitrogen fixation.

The above findings were soon followed by increasing evidence to show that nitrogen fixation in the soil was brought about by the activities of the micro-organisms present in the soil. Winogradsky [1893] isolated a new anaerobic organism from the soil, *Clostridium pasteurianum*, which was found to fix nitrogen in the deeper layers of the soil. A more important discovery in this direction was that of *Azotobacter chroococcum* and *Azotobacter agilis* by Beijerinck [1901]. These organisms were isolated from soils and canal waters

and found capable of vigorous nitrogen fixation. Thus in the course of a few decades the process of nitrogen fixation in the soil by micro-organisms became an established fact.

The most important conclusion respecting biological nitrogen fixation, which was arrived at by the beginning of this century, was that there are generally two classes of micro-organisms that fix nitrogen in the soil; the one class, the non-symbiotic or the free living, having the inherent capacity of fixing atmospheric nitrogen in their bodies, and the other class, the symbiotic, working in combination with plants belonging to the natural order Leguminosae. Subsequent researches in this field have shown that the faculty of using molecular nitrogen is also shared by a few other higher forms of life.

The role of micro-organisms in the fixation of nitrogen and its consequent influence on soil fertility and crop production was soon recognized. Since then a large volume of literature has grown up around this subject, but our knowledge of the biochemical changes that lead to the fixation of nitrogen is still meagre. An attempt is made in this paper to discuss the more important work done during the past 40 years on this problem.

#### NITROGEN FIXATION IN FREE-LIVING BACTERIA

Among the micro-organisms in which the power of fixing nitrogen is manifested, the free living bacteria constitute the most important group. They occur generally in soils and grow under the same environmental conditions as the other soil bacteria; but unlike the latter, when an adequate supply of nitrogen is not available in the medium, they have the power of building their body proteins from the nitrogen of the atmosphere. Two distinct groups have been recognized in this class of non-symbiotic bacteria, the aerobic and the anaerobic. *Azotobacter* and *Clostridium* are the typical of these two groups of organisms, which are widely distributed in nature. Owing to the fact that *Azotobacter* can be easily isolated from the soil and that it fixes comparatively large amounts of nitrogen in artificial media, this organism has been studied to the greatest extent.

#### *Occurrence, morphology and structure of Azotobacter*

*Azotobacter* is generally present in soils whose pH is not lower than 6 [Gainly, 1925] and is occasionally found to occur in the ocean and many fresh waters along with algae and other plankton organisms [Keutner, 1905]. Bergey [1930] classifies them into six distinct species, of which four only are frequently met with in the soil, *A. chroococcum*, *A. agile*, *A. vinelandii* and *A. beijerinckii*. He defines them as: 'Relatively large rods or even cocci, sometimes almost yeast-like in appearance, dependent upon the oxidation of carbohydrates. Motile or non-motile. When motile, with a single or a tuft of polar flagella. Obligate aerobes usually growing in a film upon the surface of the culture medium. Capable of fixing atmospheric nitrogen when grown in solutions containing carbohydrates and deficient in combined nitrogen'.

A medium containing dipotassium hydrogen phosphate (0.2 gm.), magnesium sulphate (0.2 gm.), sodium chloride (0.2 gm.), calcium sulphate (0.1 gm.), ferrous sulphate (0.01 gm.), sodium molybdate (0.05 gm.) and manganese sulphate (0.05 gm.) along with 5 gm. of calcium carbonate and 10 gm. of sugar in a litre of media is used for isolation and study of the bacteria.

The cells of the different species vary in size, shape and other morphological characteristics. Bonazzi [1915] has found that *Azotobacter* cells present a complex nature and different stadia of cytological make-up. The granules are of metachromatic nature and seem to have no relation to the reproduction of the cell. Lewis [1937] has distinguished volutin bodies, fat and metachromatic granules inside the cell. He has also shown that the symplasm is composed of gum. Lohnis and Smith [1913, 1923] have suggested a definite life-cycle in its mode of reproduction, but adequate evidence on this point is still wanting. This organism differs from the schizomycetes in its morphological and cultural characteristics but is closely related to yeasts [Nicol, 1933 ; Riccardo, 1925]. The role of the various cell constituents, more particularly the characteristic cell inclusions, in nitrogen fixation is not well understood. From an interesting biological study, using the respirometer technique, Iwaski [1930] has shown that on nitrogen-free medium cell multiplication may occur without nitrogen fixation, and conversely nitrogen fixation without cell multiplication. Whatever may be the underlying causes, the dissociation of nitrogen fixation from cell multiplication and the possibility of obtaining storage of nitrogen compounds accompanied by increase in cell size without increase in numbers is an interesting phenomenon not before demonstrated. He has also distinguished three different phases : multiplication phase, fixation phase and storage phase as its cultural characteristics.

#### *Metabolism in Azotobacter*

(i) *Nutritional requirements.*—The food requirements of this organism consist of a source of energy, supply of oxygen, water and certain minerals. A variety of carbon compounds, particularly the sugars, can serve as energy source for this organism. However, it is generally found that mannite is most efficiently utilized for nitrogen fixation. Conflicting views are held regarding the relative nitrogen-fixing efficiencies of other carbohydrates [Krainskii, 1908 ; Stranak, 1908]. The salts of a wide range of organic acids are also used as an energy source by this bacteria [Guittonneau and Chevalier, 1936] and among these the sodium salts of lactic and benzoic acids are found to be better energy sources for fixing nitrogen. There was nearly a constant ratio between the amount of nitrogen fixed and the heat of combustion of the fatty acids. Kuba [1930] has reported that the fatty acids of even number of carbon atoms are more useful for nitrogen fixation than the odd ones. In concentrations of the order of 0.05 per cent phenol in the medium, the organism fixes 9-11 mg. of nitrogen per gm. of phenol oxidized [Guittonneau and Chevalier, 1936]. The organism also uses the lower alcohols (for example ethyl alcohol) as a source of energy for nitrogen fixation [Mockeridge, 1915]. Some of the typical results obtained by using different carbon compounds are given in Table 1 [Mockeridge, 1915].

It may be noted that in the above experiments the efficiency of nitrogen fixation is calculated from the amount of nitrogen fixed per gram of energy material added, without taking into consideration the amount of carbon that may have been utilized by the organism. It would therefore be useful to find out the nitrogen-fixing efficiencies of the different carbon sources as a ratio, N fixed/C utilized  $\times$  time. The probable relationship between this

efficiency and the nature and configuration of the different carbon compounds used may throw light on the role of these compounds in nitrogen fixation.

Ranganathan and Norris [1927] have shown that within certain limits the amount of nitrogen fixed is proportional to the concentration of sugar present in the medium. Bonazzi [1921, 1924] differentiated between 'ferment power' or first stage in the growth of the organism when nitrogen assimilation is at a maximum and the second or maintenance phase. During the second stage the carbohydrates are actually reworked, partially burnt to liberate energy and partially utilized in the building up of soluble products. During the early periods of growth a unit of cellular substance could utilize in a unit of time 5.45 units of sugar and after an incubation period of 30 days only 0.28 units of sugar. Very small concentrations of sugar of the order of 0.01 per cent are, however, utilized more efficiently for nitrogen fixation than the higher ones [Truffaut and Bezssonov, 1922].

TABLE I

Material	(n) Nitrogen fixed on 1 gm. of energy material (in mg.)	(e) Efficiency of nitrogen fixation (n) time taken in days to com- pletely use 1 gm. substance
Polysaccharides—		
Gum arabic . . . . .	6.13	0.102
Gum tragacanth . . . . .	9.13	0.457
Rice starch . . . . .	6.40	0.355
Dextrin . . . . .	6.62	0.331
Inulin . . . . .	9.76	0.610
Sugars—		
Arabinose . . . . .	9.28	0.309
Xylose . . . . .	9.08	0.332
Dextrose . . . . .	6.57	0.329
Levulose . . . . .	10.32	0.573
Galactose . . . . .	6.20	0.282
Sucrose . . . . .	7.28	0.332
Maltose . . . . .	7.55	0.444
Lactose . . . . .	3.39	0.099
Alcohols—		
Methyl . . . . .	2.10	0.050
Ethyl . . . . .	4.02	0.119
Propyl . . . . .	9.20	0.417
Isobutyl . . . . .	4.69	0.065
Ethylene glycol . . . . .	16.74	0.492
Glycerol . . . . .	5.00	0.089
Mannitol . . . . .	11.62	0.726
Acids—		
Malic . . . . .	5.19	0.325
Tartaric . . . . .	4.54	0.162
Succinic . . . . .	8.60	0.430
Malonic . . . . .	5.32	0.266
Mucic . . . . .	6.79	0.170
Lactic . . . . .	12.01	0.706

It is of interest to note that the utilization of energy material in relation to growth and nitrogen fixation by *Azotobacter* is unique. The quantity of nitrogen fixed for every 100 gm. of glucose utilized has not been more than 1 gm. even under the most favourable conditions; whereas under thermodynamically ideal conditions when all the energy of sugar decomposition is used for fixation only 1.34 mg. of glucose is necessary for fixing 1 mg. of nitrogen. Burk has also shown that whether it fixes nitrogen or not, the organism uses the same amount of carbohydrate to produce 1 gm. dry weight of cell substance. He has found that in low oxygen tensions, *Azotobacter* uses the carbohydrates more efficiently for nitrogen fixation. At 0.001 atm. oxygen the organism uses only 1 gm. of sugar to produce 1 gm. dry weight of cell substance, whereas in most aerobic organisms, such as yeast and bacteria, 5-10 gm. of sugar is used to produce 1 gm. dry weight of cell substance. Perhaps, *Azotobacter* normally uses oxygen greatly in excess of its metabolic needs and its efficiency is therefore raised by decreasing its oxygen consumption and increasing its growth rate. It would appear from the above that although consumption of a large quantity of carbohydrate occurs during fixation, it need not be because of nitrogen fixation. The role of carbohydrate in nitrogen fixation is still awaiting solution.

Celluloses and hemicelluloses are not directly utilized by *Azotobacter* for nitrogen fixation; but these can serve as food material when the organism is grown in association with cellulose-splitting organisms [Koch and Seydel 1912; McBeth, 1914; Tuorila, 1938; Deehm, 1932; Buckstag, 1936; Skinner, 1930]. Vandecaveye *et al.* [1934] have shown that the soil organic matter is also utilized by this organism for nitrogen fixation. Lipman and Teakle [1925] have found that the soil solution is more potent than the residue.

Soil humus in small doses exerts a stimulatory influence on the organism for nitrogen fixation [Voicu *et al.*, 1930]. The effect is proportional to the concentration up to a limit, the optimum being 50 p.p.m. Iwaski [1930] and Voicu [1930] have suggested that the stimulation of nitrogen fixation by humus is probably due to the lowering of oxygen tension and consequent slowing up of oxidation and that the effect may be more on assimilation of nitrogen than fixation. Burk *et al.* [1932] explained the effect as due to the presence of iron in a more suitable organic combination in humus.

Itano [1925] and Bottomley [1920] have shown that vitamin B<sub>1</sub> and phytonucleic acid present in peat stimulate growth and nitrogen fixation by *Azotobacter*; and a number of other workers [Reed and Williams, 1915; Mockeridge, 1915; Hunter, 1922; Guittonneau and Chevalier, 1936] have reported that the presence of chemicals such as allantoin, urea, esculic acid and quinic acid depress nitrogen fixation.

In addition to energy supply some minerals in optimum concentration are also found to be necessary for the growth of *Azotobacter*, and a few among these are specific for nitrogen fixation. The presence of manganese in available form is found to stimulate nitrogen fixation; Gregario [1916] and Rocasolana [1938] have found that it accelerates the process in increasing concentrations upto an optimum limit of 0.0006 Mn ion per 100 c. c. when three times as much nitrogen is fixed as in its absence. Bortels [1930, 1936] and Nilsson [1936] have reported that minute quantities of molybdenum and

vanadium exert a definite influence on nitrogen fixation, probably they act as catalysers. Concentrations of 1 in 50 million molybdenum and 1 in 100 million vanadium increase nitrogen fixation by *Azotobacter* hundredfold; and the presence of Wolfram somehow increases their favourable effect. Molybdenum has no influence on the growth of this organism in combined nitrogen [Burk and Horner, 1936, 2], if  $N_2$  gas is absent from the medium. From this and other physico-chemical evidence adduced by Burk [1934] it is clear that molybdenum (replaceable by vanadium) is specific for nitrogen fixation.

Phosphorus compounds have been found to accelerate the activities of this organism, but the quantities required are very small; 1 p.p.m. is sufficient to permit growth and nitrogen fixation in this organism [Horner and Burk, 1934].

Horner and Burk [1934] have made a detailed study of some of the other minerals necessary for growth and nitrogen fixation in *Azotobacter*. The results are given in Table II.

TABLE II

Mineral	Mineral requirements of the organism (in m. mol)	
	In free nitrogen	In combined nitrogen
Calcium . . . . .	$2-5 \times 10^{-2}$	Negligible
Magnesium . . . . .	$2-6 \times 10^{-2}$	$2-6 \times 10^{-2}$
Iron . . . . .	$1.1-1.6 \times 10^{-2}$	$1.1-1.6 \times 10^{-2}$

It is clear from the results that the requirement of calcium is quite characteristic of nitrogen fixation in *Azotobacter*, since the need for this element is negligible when the organism is not fixing nitrogen; it has also been shown that calcium can be replaced by strontium [Burk and Lineweaver, 1931; Burk, 1932; Horner and Burk, 1934]. It would appear from the above that the element calcium or strontium has a definite role in the mechanism of nitrogen fixation by *Azotobacter*.

Iron has a favourable influence on the fixation of nitrogen by this organism [Kaserer, 1911; Sohngen, 1913; Remy and Rosing, 1911; Blom, 1931]. But the more recent evidence on this point would show that apart from the stimulatory effect that iron exerts on respiration, it plays no specific role in the mechanism of nitrogen fixation [Burk, 1934].

In addition to these, a number of other elements have also been studied in this connection. Among these mention may be made of the uranium compounds [Kayser, 1921, 2; Kayser and Delaval, 1924, 1925; Stoklasa *et al.*, 1928]; oxide, nitrate, acetate and the naturally occurring uranium mineral from Belgian Congo are found to exert a marked influence on nitrogen fixation by this organism; thus a concentration of 1 in 50,000 of uranium

in the medium increases nitrogen fixation 30-100 fold. Further work is necessary to explain the marked accelerating effect of the minerals, especially compounds of manganese and uranium on nitrogen fixation.

(ii) *Respiration*.—The respiration of this organism is dependent on the presence of sugar in the medium, being 10-15 fold more in its presence than in its absence. Stoklasa [1906] was the first to observe that *Azotobacter* breathes at an enormously high rate. The relation between oxygen pressure and oxygen uptake in *Azotobacter*; thus the rate of respiration is maximum at 0.15 atmos. and diminishes on each side of this value being 1/3 at 0.005 atmos. and also at 1.0 atmos., the decrease between 0.2 and 1.0 atmos. being linear. The changes are immediate and reversible. This is in striking contrast to most of the cells and tissues hitherto studied; the respiration of yeast for example being independent of oxygen pressure between 0.03 and 0.97 atmos. The respiration per unit dry weight of the organism has a value  $QO_2$  2000 [Meyerhoff and Burk, 1928], whereas it is 1/25 this value in other forms of bacteria. This rate subsides markedly with increasing age of the culture medium. Narcotics and HCN act similarly on respiration rate as with other cells. From calorimetric studies Fife [1931] has shown that the rate of respiration remains constant over long periods and that during nitrogen fixation the O/N ratio effecting maximum respiration is the same as when the organism grows in a nitrate medium. The results being not in full accord with those of Meyerhoff and Burk, further confirmation is necessary before any definite conclusions can be drawn from his results.

Burk *et al.* [1932, 2] have shown that respiration is a reversible function of pH (optimum at 7.2 and limits at 5 and 9) and temperature (optimum at 34 and limits at 10 and 50). The temperature characteristic of respiration for *Azotobacter vinelandii* is  $19.330 \pm 0.115$ . Lack of carbohydrate or oxygen causes little permanent injury to respiratory enzyme. Catalase activity is optimum at neutrality but is insensible to acidity or elevated temperature.

Spectroscopic examination of *Azotobacter* cells reveals a respiration mechanism similar to that ascribed to aerobic cells. Keilin [1933] has shown that the Fe compound is the O transporting enzyme and the  $Fe^{++}$  and  $Fe^{+++}$  being the cytochrome components. With oxidation there is a shift of the band from 632 to 647. A band at 630 which fades on shaking with  $O_2$  and reappears on reduction is due to  $Fe^{++}$  component in the pigment.

Negelin *et al.* [1934] have studied the shift of bands in relation to narcotics. In spite of its high respiratory quotient, the O transporting enzyme is not affected by carbon monoxide [Keilin, 1933]. The respiration rate is sensitive to HCN and Cu; with the former the effect is reversible, but with the latter the Cu ion forms a solid compound with the cell substance. The effect of Cu on respiration is more marked when the culture is placed for a short time under  $O_2$  deficiency than those having plenty of  $O_2$ .

The respiratory enzyme band being in the red region at 632 instead of at yellow, is unique in *Azotobacter*. Such bands are never found to occur in the pheohemins, but occur in the group of compounds which result when Mg of chlorophyll is replaced by Fe. In view of the close similarity of the enzyme band with that of chlorophyll, it is probable that this enzyme plays

an important part in the fixation of nitrogen in the same manner as the chlorophyll does in the fixation of carbon during photosynthesis. Further work on these lines would lead to results of great value in understanding the mechanism of nitrogen fixation.

(iii) *Environmental conditions*.—The activity of *Azotobacter* and its efficiency for nitrogen fixation are also dependent on the reaction of the media, temperature and air supply.

In general a reaction below pH 6.0 and above pH 9.1-9.6 is not favourable to the growth of *Azotobacter* [Wenzl, 1934, 2; Martin and Brown, 1938]. The optimum for growth is very near the optimum for fixation [Gainey and Batchelor, 1922; Gainey, 1923] and is different for the different species [Endres, 1934; Willis, 1933, 2].

TABLE III

Species	Optimum pH	Limiting pH
<i>Chroococcum</i> . . . . .	7.45-7.60	5.8
<i>Beijerinckii</i> . . . . .	6.65-6.75	5.8
<i>Vinelandii</i> . . . . .	7.50-7.70	5.9

Thus the activity of the organism in the soil is inhibited by acid root secretions from the roots of plants such as maize and wheat [Shelonmova and Menkina, 1935]. Starkey and De [1939] have isolated a new species of *Azotobacter* from soils of India which are acid in reaction (pH 4.9-5.2). Alston [1936] has also reported the presence of a unique strain of *Azotobacter* in Malayan soils, which can tolerate pH 3.6.

There is a marked influence on the amount of nitrogen fixed per gm. of mannitol oxidized when the cultures of *Azotobacter* are exposed to light of different colours; in general yellow light is better than blue [Kayser, 1921, 1; Itano and Matsuura, 1935]. The fact that the organism fixes nitrogen even in darkness shows that the light does not play any fundamental role in the fixation of nitrogen; nevertheless it contributes in some way towards accelerating the process. It would be of interest to find out the mechanism by which light of different wave-lengths thus stimulates nitrogen fixation. It has been claimed by Stoklasa *et al.* [1928] and Kayser and Delaval [1924, 1925] that exposure to radio-activity increases nitrogen fixation by *Azotobacter*; in the former case by passing a current of activated air through the culture and in the latter by addition of a powdered radio-active mineral. Stoklasa and Kricka [1928] have shown that  $\beta$  and  $\gamma$  rays depressed the transformation of nitrogen into nucleo-proteins and albumins in the cells of *Azotobacter*, while radium emanation has the opposite effect.

The organism grows and fixes nitrogen between temperatures 10°C. and 50°C., the optimum being 34-35°C. This value is dependent on pH and O<sub>2</sub> tension [Lineweaver, 1933]. Omeliansky [1915, 1926] has shown that

the organism can withstand long periods of drought. It has been recorded that nitrogen fixation by *Azotobacter* in tropics takes place at 35°C. [Dhar and Tandon, 1936] and in the Arizona soils at 32·5°C. [Greene, 1932].

Aeration of the medium facilitates nitrogen fixation by *Azotobacter* [Ashby, 1907; Hunter, 1922]; thus in a sand medium the organism fixes more nitrogen than in liquid medium [Krainskii, 1910, 1912]. This may be due to quicker oxidation of the sugar, which from the point of view of efficiency may not be necessary.

The presence of other organisms in the medium in which *Azotobacter* grows has been found to exert a stimulating influence on nitrogen fixation by this organism. Thus, in the presence of (a) bacteria, *granulobacter*, *aerobacter*, *radiobacter* and *psuedomonas radicola* [Beijerinck and Von Deldon, 1902; Bottomley, 1910], (b) protozoa, *amoeba* [Kovats, 1928; Vinogradova, 1928] and (c) algae such as *oscillaria*, *gleoscapsa* and *chlorella* [Menechikovosky, 1933]. *Azotobacter* fixes more nitrogen. Three times as much nitrogen is fixed in presence of *aerobacter aerogenes* [Kalantarian and Panassian, 1930] and in presence of *pseudomonas radicola* there is two-fold increase in the amount of nitrogen fixed per gm. of sugar consumed [Bottomley, 1910]. Hanzawa [1914] has found that mixed cultures of different species of *Azotobacter* possess a stronger nitrogen-fixing power than the individual strains.

(iv) *Influence of combined nitrogen on nitrogen fixation.*—The study of the fixation of nitrogen in media containing combined nitrogen has shown that fixation of elementary nitrogen is resorted to by the organism only in the absence of sufficient amounts of available combined nitrogen in the medium [Zoond, 1926; Fuller and Rettiger, 1931], the equilibrium concentration of rapidly available combined nitrogen required to inhibit nitrogen fixation is 0·5 mg. per 100 c. c. [Burk and Lineweaver, 1930]. From a study of the nitrogen changes produced by *Azotobacter* in media containing different nitrogen compounds, Thompson [1934] has shown that they are first converted into ammonia before they are utilized by the organism.

By repeated culturing of *Azotobacter* in a medium containing sodium benzoate it has been possible to evolve a strain of the organism which can fix nitrogen even in the presence of combined nitrogen in the medium [Remzer, 1938]. The nitrogen-fixing capacity of the organism is suppressed by growing it for long periods in media containing potassium nitrate [Stumbo and Gainey, 1938]. In this connection it would be of great interest to study the change in the azotase system under the above methods of culturing the organism.

(v) *Products of metabolism.*—In dextrose media *Azotobacter* produces formic, acetic, lactic and tartaric acids and ethyl alcohol, a large part of it being converted into carbon dioxide [Ranganathan and Norris, 1927; Aso *et al.*, 1932]. Pthalic acid has also been detected in *Azotobacter* cultures [Aso, *et al.*, 1932]. Siffred and Anderson [1936] have found that a mixture of sterols allied to ergosterols and the water-soluble vitamin B<sub>1</sub> are elaborated by this organism in culture medium. As compared with other types of soil bacteria there is nothing unique in the products of metabolism of this organism.

The various physiological functions (respiration, growth and efficiency) being quite similar when the organism grows in free or combined nitrogen, no conclusion can be drawn from these on the chemical mechanism of nitrogen fixation.

#### *Chemical analysis of Azotobacter cells*

In general the composition of the cells does not vary greatly from that of other common bacteria. The cells consist chiefly of carbohydrates, proteins and a small percentage of minerals. The proportion of these various constituents vary with the different species as also with the physical condition of the medium in which the organism is grown [Hoffman, 1913; Omeliansky and Seiber, 1913].

(i) *Nature of proteins*.—A typical analysis of the cells with special reference to the proteins [Greene, 1935] is given in Table IV.

TABLE IV

Species	As percentage on dry cell material			As percentage on total			
	Carbo-hydrate	Protein	Ash	Amide N	Humin N	Basic N	Filtrate N
<i>A. chroococcum</i>	62.34	25.00	4.00	17.90	9.62	20.00	52.50
<i>A. beijerinckii</i>	64.72	24.68	4.05	18.25	13.67	25.94	42.03
<i>A. agile</i>	22.09	61.19	7.55	13.42	13.18	26.90	45.77
<i>A. vinelandii</i>	25.67	52.25	7.66	20.36	15.82	22.22	41.50

The Van Slyke analysis of the proteins have shown that in general the cells contain a large amount of arginine varying from 16 to 20 per cent.

It is evident from the table that there is a close similarity between *chroococcum* and *beijerinckii* on the one hand and *agile* and *vinelandii* on the other with regard to their general chemical composition. Although more of carbohydrate is synthesized by the first two, they fix comparatively less amount of nitrogen than *agile* and *vinelandii*. It would appear from the above that carbohydrate elaboration in the cells of these organisms has no significance in nitrogen fixation.

Hoffman and Hammer [1910] have found that the amount of nitrogen in dry cell material increased as the incubation period advanced, being 1.88 per cent in seven days and 2.84 in 21 days. This shows a very high content of non-protein material which though not in agreement with Stoklasa was confirmed later by Omeliansky and Seiber [1913].

It has been found that the proteins of *Azotobacter* form a better source of nitrogen for alcoholic fermentation [Kayser, 1921, 1]. The close similarity in the amino acid make-up of *Azotobacter* proteins and those of leguminous plants [Greaves and Reeder, 1935] would suggest that there is some parallelism in the fixation and assimilation of nitrogen in these two systems. A comparative study of the proteins formed by this organism when grown in

media containing free and combined nitrogen has not so far been attempted. A study of the change in the complexity factor of the proteins of *Azotobacter* at various stages of growth and nitrogen fixation in these media would throw light on the chemical steps through which the protein is synthesized in this organism.

(ii) *Slime production*.—When grown in culture media this organism produces a characteristic slime; the slime is essentially a carbohydrate, levorotatory, resistant to hydrolysis by acids, and belongs to the class of true gums [Sanborn and Hamilton, 1929]. The production of gum varies in the different species and is increased by having more complex carbohydrates in the medium [Hamilton, 1931].

The role of slime in the mechanism of nitrogen fixation is not very clear. Bhaskaran [1936] has shown that the cells and slime of *Azotobacter* exhibit definite and unique C/N relationships during the growth of this organism in culture media. The greater intake of carbon by the cells and slime production during the early stages of growth would suggest that the formation of slime is a necessary precursor for nitrogen fixation which follows later on. A study of the change in C/N of the bacterial cells freed from slime would throw further light on this point.

Burk [1934] has observed that high nitrogen pressure is necessary for the enzyme catalysis resulting in the fixation of nitrogen; thus a concentration of  $1.6 \times 10^{-4}$  M of nitrogen is necessary in the medium for successful enzyme action. The solubility of nitrogen gas in water being very low, it is probable that the slime may serve as an efficient medium for adsorbing the nitrogen gas before it is fixed. In view of this, it would be useful to study the solubility of nitrogen gas in the slime. The production of slime when the organism grows in a medium containing combined nitrogen (when the organism fixes no N) is also a point of great interest.

(iii) *Pigment formation*.—With ageing in the culture medium the different species of *Azotobacter* form characteristic pigments: *A. chroococcum* forms a brown pigment, *A. agile* a fluorescent dye and *A. vinelandii* an yellow, water-soluble greenish fluorescent pigment similar to the flavone pigments [Petschenko, 1930; Ellinger and Koschara, 1933]. Pigment formation is greatly affected by the nature of the nutrients present in the culture media: the presence of dextrin, chalk,  $\text{Ca}(\text{NO}_3)_2$  and increased air supply facilitate its formation; and traces of  $\text{MnSO}_4$ ,  $\text{ZnSO}_4$  and exposure to ultra-violet rays induce early pigmentation and shorter life in the organism [Omeliansk yand Seuerova, 1911; Hills, 1918; Colley, 1931; Itano and Matsuura, 1935]. In the case of *A. vinelandii*, pigment formation takes place only in presence of glycerine in the medium. The presence of small amounts of acetone in *A. chroococcum* and sodium acetate in the case of *A. agile* inhibits pigment formation [Wenzl, 1934, 1].

The pigment is a melanin of unknown nature formed from tyrosine by the action of tyrosinase [Umgerer, 1934]. The significance of this characteristic pigment formation in the life of the organism is not very clear. Probably with advancing age and death of the organism, conditions in the cell are made more favourable for the action of the tyrosinase present in the system to convert tyrosine into melanin, thereby conserving the nitrogen in the more resistant form in nature.

*Biochemical mechanism of nitrogen fixation in Azotobacter*

Our knowledge of the biochemical mechanism by which the organism fixes nitrogen is still meagre. The work done so far in this direction can be conveniently dealt with under the following heads :—

(i) *First demonstrable chemical step in nitrogen fixation.*—Various nitrogenous compounds have been detected in *Azotobacter* cultures fixing nitrogen and these have been supposed to be the first intermediate product in nitrogen fixation.

Gautier and Drouin [1888] and Boneima [1903] were first to suggest that fixation takes place by direct oxidation of the free nitrogen to nitrate. A few workers have also detected nitrate in cultures; but it has been shown that the phenoldisulphonic acid test for nitrates which these workers have adopted is not reliable because of the presence of pigments [Kellerman and Smith, 1912]. A number of workers have also subsequently failed to detect the presence of nitrates or nitrites in the medium in which fixation took place. Direct experimental proof in support of oxidation of nitrogen to give oxides,  $\text{NO}_2$  and  $\text{NO}_3$ , is still lacking.

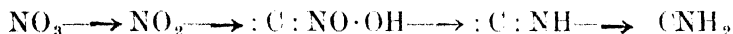
The presence of ammonia has been detected in *Azotobacter* cultures by a number of workers and this is generally considered to be the first demonstrable stage in nitrogen fixation [Kostychev and Ryskaltehou, 1925; Ranganathan and Norris, 1927; Halverson, 1927]. Wieland [1922] considered that the action of hydrogen acceptors formed in the cells of nitrogen-fixing bacteria does not depend upon oxygen for hydration but upon molecular nitrogen with which it forms ammonia perhaps through the hydrazine stage in a manner similar to Haber process. Winogradsky [1930, 1, 2] has demonstrated the formation of ammonia by an ingenious culture technique, using alkali salts of organic acids as nutrient for the organism. Kostychev *et al.* [1926] have adduced evidence to show that the fixation in *Azotobacter* is an extra-cellular reduction process and Winogradsky [1932] has further suggested that the ammonia is formed by the action of an enzyme—a dehydrogenase—with the aid of a catalyser, perhaps by symbiotic action. Kostychev *et al.* [1926] and other workers have confirmed these findings. By mixed culture studies of *Azotobacter* and other ammonia utilizing organisms, like mycoides, in nitrogen-free media, Novogradskii [1933] has adduced additional evidence for the formation of small quantities of ammonia in the medium.

This question of ammonia formation has attracted much attention in recent years. Burk and collaborators [1935, 2; 1936, 1] have critically examined the origin of ammonia detected by these workers and have come to the conclusion that this is due to the secondary oxidative de-amination of the bacterial protein which takes place in the medium side by side with nitrogen fixation. Isakova [1933] has shown that the amount of ammonia formed in the presence of glucose remains fairly constant with negligible variation, but increases after glucose disappearance and that this ammonia production is more marked in acid cultures due to autolysis of cells. But, on the other hand, a number of workers have reported that this secondary ammonia formation does not take place in presence of sugar in the medium. In view of the above conflicting evidence it would be difficult to draw any definite conclusion in regard to the origin of ammonia in the cultures.

By means of *in vitro* experiments using Pt and Pd as catalysts Knoop [1927] has suggested that where ketonic acids and ammonia meet in the cell under conditions favourable for hydrogenation, amino acids are formed very readily.

Hydroxylamine, hydrazine and amide have been reported from time to time as the first intermediate stage in nitrogen fixation, and among these the hydroxylamine theory needs some consideration. Blom [1931] from theoretical considerations has suggested that hydroxylamine is first formed with Fe as catalyst. In the light of this theory he has explained the checking action of  $\text{NO}_3$  and  $\text{NH}_3$  on nitrogen fixation. The conclusion arrived at by Burk regarding the non-specificity of Fe in fixation would suggest that Blom's idea of iron catalysts requires revision.

Recently Endres [1934, 1, 2] has adduced further evidence in support of the hydroxylamine theory. In lactate cultures he has detected 1.2  $\gamma$  of hydroxylamine. He has shown that this is formed by the hydrolytic decomposition of oximes. In *Azotobacter* cultures fixing nitrogen the oxime concentration increases from 0.5 to 12ml/litre in 72 hours [Endres, 1934, 3], whereas in the absence of elementary nitrogen there was no increase in the oxime concentration. By studies in nitrate cultures, Endres and Kauffmann [1938] have further suggested a scheme of formation of oximes and amino acids from  $\text{NH}_2\text{OH}$  as follows :



On the other hand, Burk and Horner [1935, 1] have reported that the compound  $\text{NH}_2\text{OH}$  and oximes beyond 3 mg./litre are toxic to the growth of *Azotobacter* and that  $\text{NH}_2\text{OH}$  is not produced in nitrate cultures.

Direct union of nitrogen with carbon compounds to form amino acids has also received some attention in recent times. A number of workers [Waynick and Woodhouse, 1922; Halverson, 1927; Kumagawa, 1928] have detected amino acids in *Azotobacter* culture media. Among these the observations of Virtanen and Laine [1938] are interesting. By analogy from legume fixation and as a result of direct experiments with *Azotobacter*, he has suggested that aspartic acid is formed before ammonia in the medium, the aspartic acid being formed through the oxime of oxal-acetic acid. Direct experimental evidence of its formation has been adduced by analysis of copper salt of the oxime and oxyacids have also been detected in cultures. The aspartic acid thus formed later on yields ammonia. This theory agrees with the oxime theory of Endres and also accounts for the ammonia formed in culture media. From the evidence so far available it would appear that this is the most probable mechanism by which the  $\text{N}_2$  molecule is fixed by the free-living bacteria, nevertheless conclusive evidence on this point is still wanting.

(ii) *Enzyme systems*.—Bakh [1934] made a sensational announcement that the liquids containing the enzyme obtained by filtering *Azotobacter* cultures at 300 atmospheres through Chamberland  $\text{L}_3$  candles fixes atmospheric nitrogen in presence of sugars. His results are presented in Table V.

The results show fixation of nitrogen in cell-free cultures. This observation has not been further confirmed. The present authors have obtained

evidence (unpublished data) to show that cells treated with antiseptics, chloroform and toluene, fix considerable amounts of nitrogen in sugar media.

TABLE V  
*Amount of nitrogen fixed by Azotobacter cultures*

Time in days	Nitrogen in mg.		
	Control	Glucose	Mannitol
0 . . . . .	1·976	..	..
9 . . . . .	2·783	14·683	10·645

Certain amount of definite information is available regarding the properties and behaviour of the enzyme system fixing nitrogen in *Azotobacter* cells, as a result of the researches of Burk and co-workers. They have studied the enzyme system by means of the well-known Warburg manometric technique using living cultures of the organism. The studies have been made with special reference to the auxillary substances and environmental conditions necessary for the enzyme action. The properties of the enzyme have been defined on the basis that the matabolic activities of the organisms obtaining their nitrogen from nitrogen gas are specifically ascribed to the fixation process and that the organisms obtaining nitrogen from combined nitrogen do not behave similarly.

Burk and co-workers [1934, 1, 2, 3] have considered the enzyme system fixing nitrogen in *Azotobacter* as a phyto—enzyme (an enzyme whose activity is correlated with the structure of the living cell to the extent that the velocity of formation of the intra-cellular products parallels and is normally measured by the velocity of growth) and has named it as azotase. The specific component within the azotase system which combines with the  $N_2$  molecule is termed nitrogenase, (E). The fixation of nitrogen at ordinary temperatures and pressures by *Azotobacter*, as a function of the nitrogen pressure, corresponds to one  $N_2$  molecule combining reversibly with one enzyme molecule E (nitrogenase) to form a compound  $N_2E$  which later reacts to yield protein according to equation,  $N_2 + E \longrightarrow N_2E \longrightarrow P$  [Lineweaver *et al.*, 1934]. The thermodynamic dissociation constant of the above reaction,  $KN_2$  ( $[E][N_2]/[N_2E]$ ) has been found to be  $0.215 \pm 0.002$  atmospheres. This corresponds to  $1.64 \times 10^{-4}$  M of dissolved nitrogen gas in the medium at  $31^\circ C$ . Compared to most other enzyme reactions in which gases are involved, such as photosynthesis and respiration (where  $kCO_2$  and  $kO_2$  are of the order of  $10^{-5}$  atmospheres), a large nitrogen pressure appears to be needed for enzyme reaction.

From energy considerations and entropy they have shown that the decomposition of  $N_2E$  is a bimolecular reaction and only a negligible fraction of the total amount of carbohydrate is used for nitrogen fixation by the enzyme system under thermodynamically ideal conditions, thereby showing that

probably carbohydrate plays no role in the enzyme mechanism of nitrogen fixation. The relationship of the dissociation constant under varying conditions of enzyme action would point out that more than one active intermediate is not formed in the system. The free energy of dissociation of  $N_2E$  has been found to be 100—1,000 calories.

The dissociation constant is quite characteristic of the reaction of the medium, being independent of the following factors: concentration of calcium, oxalate, iron and indifferent narcotics such as alcohols urethanes and urea derivatives; pressure of oxygen; temperature;  $pH$ ; and certain physiological factors such as the species of *Azotobacter*, concentration and age of the culture. The concentration of calcium, strontium, oxalate and  $pH$  are known to bear a specific relation to the mechanism of nitrogen fixation as distinguished from growth. Nitrogen fixation by azotase decreases from a maximum at  $pH$  7.8 to a zero limit at  $5.97 \pm 0.02$ , and irreversible inactivation occurs only below  $pH$  5.0. The rate of decomposition of fixed nitrogen decreases from a maximum at  $pH$  7.8 to a limit at  $pH$  4.5. The rate of  $O_2$  consumption as function of  $pH$  is similar in type. The  $pH$  value for fixation is a characteristic constant independent of other reactions. Results on the study of phase changes with special reference to azotase activity in relation to  $pH$  would suggest that the nitrogen-fixing system is a two-component heterogeneous system of three phases in equilibrium at a critical  $pH$ .

They have also adduced evidence to show that Ca replaceable by strontium and molybdenum replaceable by vanadium are specific for nitrogen fixation by azotase; Fe does not play any part in the process of nitrogen fixation by azotase.

By studies in free and combined nitrogen they have further shown that the enzyme systems responsible for assimilation of free and fixed nitrogen are different although considerable relationship exists between them. A more specific nitrogen-fixing enzyme E with which  $N_2$  combines before forming  $N_2E$  is also postulated.

From a study of the influence of various nitrogen pressures on nitrogen fixation by *Azotobacter*, Burk [1930] has found that the effect is immediate and reversible, being proportional to  $N_2$  pressure. The effect is felt to an appreciable extent at 0.5 atmosphere with limits at 5—10 atmospheres.

Nitrogen fixation by azotase is also proportional to  $O_2$  tension in the medium; the efficiency ratio (nitrogen fixed/ $O_2$  consumed) increases 10 to 20 fold between 0.21 and 0.01 atm.  $O_2$  and the rate of nitrogen fixation attains a maximum at 0.04 atm.  $O_2$  being only  $\frac{1}{3}$  or  $\frac{1}{4}$  at 0.008 and at 0.21 atm. The influence of  $O_3$  is not affected by the presence of other gases such as  $H_2$  and  $N_2$ . The nitrogen obtained from *Azotobacter* suspensions by vacuum extraction at low temperatures obeys typical Henry's law relationships and are similar to those obtaining in legume bacteria and yeasts.

A certain amount of work has also been done on the other common enzymes present in *Azotobacter*. The cells are found to be rich in catalase [Burk *et al.*, 1932, 2], numerous dehydrogenases [Lineweaver *et al.*, 1932], cytochrome [Meyerhoff and Schulze, 1932; Keilin, 1933; Negelein and Gerisher, 1933], the spectroscopically observable 'atmungs ferment' [Negelein and Gerisher, 1933], malonate carboxylase and the newly observed

co-enzyme R [Allison, 1933]. Nilsson [1936] has found that in mannitol *Azotobacter* develops a dehydrogenase which is absent when the organism grows in glucose; hexose phosphate dehydrogenase is present in both cases.

The extra-cellular isolation of azotase and the study of the enzyme apart from the growth and general metabolism of the organism have not so far been possible and any advance in this direction would greatly help to understand the enzyme mechanism responsible for nitrogen fixation.

#### *Nitrogen fixation in clostridium*

(i) *Distribution of clostridium*.—Winogradsky [1893] isolated a sporogenous anaerobic organism, known as *Clostridium*, from the soil of St. Petersburg which has the power of assimilating nitrogen and bringing about the butyric fermentation of carbohydrates. He further demonstrated the wide occurrence of this organism in the soils of St. Petersburg and Paris. Omeliansky demonstrated the presence of this organism in almost all Russian soils that he examined. This was also present in most of the German soils examined [Freudenreich, 1903]. Haselhoff and Bredmann [1906] have found that in nearly all samples of soils and leaf moulds examined, *Clostridia* were present. They are found in rather large numbers in acid soils. Addition of  $\text{CaCO}_3$  or  $\text{CaO}$  to the soil had little effect on the numbers of *Clostridia* or the amount of nitrogen fixed. It is much more abundant than *Azotobacter*; the number being over 100,000 per gm. of soil [Truffaut and Bezssonov, 1921].

*Clostridium* is as important as *Azotobacter* in nitrogen fixation [Omeliansky, 1906]. Truffaut and Bezssonov [1921] have suggested that it is *Clostridium* and not *Azotobacter* that is the principal agent for the fixation of nitrogen in the soil. In the acid soils of Iowa, the anaerobic nitrogen-fixing organisms are of considerable importance in maintaining the fertility; it can be found in soils even with a pH of 5.0 — 5.2. It is of interest to note in this connection that although considerable amounts of nitrogen are fixed, there is little increase in ammonia or amino nitrogen [Walker and Willis, 1933]. The number of *Clostridia* present in the soil can be increased considerably by partial sterilization using  $\text{CaS}$  and heat.

(ii) *Morphology*.—Bergey [1930] defines them as: 'Anaerobes or micro-aerophiles, often parasitic. Rods frequently enlarged at sporulation producing *Clostridia* or *Plectridia* forms'. A few of these have the power of fixing nitrogen and *Cl. pasteurianum* is typical of the species fixing nitrogen.

Winogradsky found *Cl. pasteurianum* to be an obligate anaerobic form which can develop under aerobic conditions only in presence of aerobic bacteria. However, the facultative aerobic *Cl. americanum* isolated by Pringsheim [1906] was very similar in morphology to the *Clostridia*, but was capable of fixing large quantities of nitrogen, perhaps due to its aerobic nature.

Bredmann [1912] after examining a large number of species of *Clostridia* came to the conclusion that the various strains classified into different groups belong to one and the same species, viz., *Bacillus amylobacter*, the differences being due to the methods of isolation and treatment. It is easy to bring about a regeneration of the nitrogen-fixing capacity. The latter observation has, however, been questioned by Pringsheim.

McCoy *et al.* [1928] have studied this group of organisms and have recognized three sub-groups—*Cl. pasteurianum*, *Cl. saccharo-butyrus* and the *plectridium* type. They have found that the different groups fix varying amounts of nitrogen in culture media; *pasteurianum* fixes 0.66 - 3.98, *butylicum* 0.64 — 2.35 and *plectridium* 0.65 — 2.78. The differentiation of these is not well defined.

(iii) *Cultivation and cultural characteristics.*—A modification of Winogradsky's nutrient medium adding soil extract, small amounts of  $\text{MgSO}_4$  and  $\text{Na}_2\text{HPO}_4$  along with traces of Fe and Mn salts and sufficient  $\text{CaCO}_3$  has been found useful for the cultivation of *Clostridium*. It can be easily isolated by prolonged pasteurization at 75°C.

Itano and Arakawa [1930] have recommended a method for the culture of *Cl. pasteurianum*; the method is but a modification of the Morse-Kopeloff culture of anaerobes. Partial sterilization leads to increased nitrogen fixing power in *Clostridia*, probably due to the destruction of substances harmful to them [Truffaut and Bezssonov, 1921]. The optimum temperature for the development of *Cl. pasteurianum* is 28° — 30°C. The optimum reaction is pH 6.9 — 7.3, but the organism still develops well at pH 5.7. It has been observed that it can withstand a greater acidity than proteolytic anaerobes like *Bacterium putrificus*, from which it can thus be freed. Addition of  $\text{CaCO}_3$  to the glucose medium has a favourable effect in neutralizing acids formed and doubling the amount of nitrogen fixed [Truffaut and Bezssonov, 1922]. In this connection  $\text{MgCO}_3$  is found to be less favourable. At pH 6.5 — 9.5 *Clostridium* fixed 4 — 4.3 mg. per 50 c. c. medium in three weeks; at pH 5.0, 3.2 mg. of nitrogen was fixed.  $\text{CaCl}_2$  has also been found to be useful in promoting nitrogen fixation [Willis, 1933, 1]. Willis [1933, 2], however, has shown that pH of the medium has little effect on the amount of nitrogen fixed in the medium between pH 5.0 — 9.5. Omeliansky [1906] has found that the organisms are very active at 30°C. and fix less nitrogen at higher temperatures.

When freshly isolated, the *Clostridium* fixes more nitrogen than when cultivated for a long time in artificial media. The culture can be invigorated by growing it in Winogradsky's medium to which enough ammonium sulphate is added so as to offer the organism less nitrogen than is needed for the complete decomposition of the sugar. By transferring from this culture, when gas formation ceases, normal growth and nitrogen fixation is obtained [Bredmann, 1909]. Bredmann has further shown that the culture could be invigorated by passing it through soil.

Spores are formed when the organism is grown in medium with plenty of oxygen; the presence of 30 mg. of oxygen per litre of air will still allow spore formation. The spores are not destroyed at 75° even at the end of 15 hours; at 100° the spores are destroyed in five minutes. The spores could be preserved in the dry state for 20 years with the nitrogen-fixing power intact [Omeliansky, 1906].

(iv) *Fermentation of carbohydrates.*—The fermentation of various carbon compounds depends on the nature of the nitrogenous nourishment. Dextrose, sucrose, levulose, inulin, galactose and dextrin are fermented in presence of peptone, while only dextrose, sucrose and inulin are attacked when ammonium sulphate is present. Lactose, arabinose, starch, gum, mannitol, dulcitol,

glycerol and calcium lactate are not attacked by the organism under any conditions [Winogradsky, 1902]. *Clostridium* does not attack cellulose, while in presence of cellulose-destroying bacteria more nitrogen is fixed than with carbohydrate alone [Pringsheim, 1913]. Omeliansky [1906] has observed that glycerol, mannitol, maltose and raffinose are also fermented by this organism. Peterson *et al.* [1926] have studied the fermentative powers of *Cl. thermocellum* on nine sugars and five related compounds. Stickland [1934] has carried out experiments on the chemical reactions by which *Cl. sporogenes* obtains its energy.

The characteristics of the butyric fermentations brought about by the organism are such that 42-45 per cent of the dextrose is converted into a mixture of acetic and butyric acids in varying proportions; small amounts of alcohol, ethyl, propyl and isobutyl, are formed, and a considerable evolution of a mixture of  $\text{CO}_2$  and  $\text{H}_2$  occurs [Winogradsky, 1902]. Acetyl-methyl carbinol is formed by *Clostridium acetobutyricum* along with acetic and butyric acids. Its production can be increased by the addition of phosphates and decreased by proteins and is more closely associated with the formation of acids than that of acetone and butyl alcohol. Wilson *et al.* [1927] have shown that acetyl-methyl carbinol is a regular end product of the fermentation, it being formed at the same time as acetic and butyric acids and all three probably have the same precursor. With fermentation the acidity of the medium changes to approximately pH 3-4. Butyric acid forms the larger part of the volatile fraction of the acids [Walker and Willis, 1933]. The amount of acid produced is correlated with the amount of glucose utilized. It produces large amounts of  $\text{CO}_2$  under anaerobic conditions in a medium containing  $\text{CaCO}_3$ . The main source of carbon dioxide is the glucose molecule, though small amounts result from  $\text{CaCO}_3$  [Willis, 1933, 1].

(v) *Anaerobic nitrogen fixation*.—In nitrogen-free medium *Clostridium* fixes about 3 mg. of nitrogen per gram of sugar decomposed [Winogradsky, 1902]. Haselhoff and Bredmann [1906] have found that both crude and pure cultures of anaerobic bacteria isolated from soil absorb quite considerable amounts of nitrogen similar to that reported by Winogradsky. For each gram of sugar fermented 3-6 mg. of nitrogen was assimilated. Too large an increase in the nitrogen content of the medium decreases nitrogen fixation and finally stops it entirely, but nitrogen fixation still takes place when the ratio of N : sugar is 16 : 100, whereas according to Winogradsky this ratio is 6 : 1000. *Cl. acetobutylicum* (Weizman) fixes comparatively little nitrogen; the four strains studied fixed from 0.69 to 1.06 mg. in 100 c. c. medium. From a study of single cell culture of *Clostridium*, McCoy *et al.* [1928] have correlated nitrogen assimilation with consumption of sugar although the relative efficiency varies with the stage of growth. The greater the concentration of sugar the lower is its economic utilization, 3.2 mg. of nitrogen being fixed per gram of glucose in 0.5 per cent solution, 2 mg. in 2 per cent solution and 1.2 mg. in 4 per cent solution [Waksman, 1931].

Combined nitrogen in the medium reduces nitrogen fixation,  $\text{NaNO}_3$  having the least effect. No nitrogen is fixed in the presence of ammonium sulphate and in peptone only 0.6 mg. of nitrogen is fixed in 25 days. The production of  $\text{CO}_2$  is directly associated with the sugar utilization, the amount

being the same whether the organisms are grown in an atmosphere of nitrogen or air [Walker and Willis, 1933 ; Willis, 1933,2]. Glucose was rapidly used in medium containing peptone, ammonium sulphate or  $\text{NaNO}_3$ , but little or no nitrogen was fixed.  $\text{CaCO}_3$  may act as a neutralizer or an acceptor for H in anaerobic fermentations [Willis, 1933,1]. In media containing 0.2  $\text{CaCl}_2$  or 15 gm. of  $\text{CaCO}_3$  per litre comparatively large amounts of nitrogen are fixed in the solution.  $\text{NO}_2$  and  $\text{NO}_3$  are formed in presence of  $\text{CaCO}_3$  but not in culture media containing  $\text{CaCl}_2$  [Willis, 1933,2]. When the solution contains more than six parts of combined nitrogen in one thousand parts of solution, nitrogen fixation comes to a stand still. Omeliansky has, however, obtained some fixation even with a concentration of 16 parts of combined nitrogen in the medium.

Comparatively more of carbohydrate is used for nitrogen fixation by the anaerobic bacteria. But if the energy liberated rather than the carbohydrate used is taken into consideration, the anaerobic species are as efficient as the aerobic ones from the point of view of energetics of nitrogen fixation.

#### *Nitrogen fixation in other free-living bacteria*

It has been claimed from time to time that a large number of organisms in the soil, other than *Azotobacter* and *Clostridium*, have the power of fixing nitrogen [Greaves, 1929, 1930 ; Emerson, 1917].

Lichtenstein *et al.* [1907] have described a new aerobic nitrogen-fixing *Clostridium* having only about half the capacity of the previously described *Clostridia*. Truffaut and Bezssonov [1925, 1] have isolated a new bacillus from soil which fixes 2.7 mg. of nitrogen per gm. of carbohydrate, *Bacillus Truffanti*. Lipman [1938] has isolated some new non-symbiotic bacteria from the white sands of New Mexico, which have the power of fixing nitrogen.

#### SYMBIOTIC FIXATION OF NITROGEN BY NODULE BACTERIA

Boussingault [1838] pointed out that leguminous plants absorb atmospheric nitrogen, basing his conclusion on the experiments he conducted with clover and wheat. But not until 1888 it was known by the classical researches of Hellriegel and Wilfarth that these plants could gain atmospheric nitrogen through the nodules present in their roots. They showed that nitrogen is fixed because of the presence of a species of bacteria known as *Bacillus radicola* in the nodules. Following up this important piece of work, Beijerinck succeeded in isolating this organism from the plant, and attempted to study the symbiosis between the bacteria and the host plant. Since then a large volume of work has been done on this bacterium, but our knowledge of symbiotic nitrogen fixation is still meagre.

#### *Biology of legume bacteria*

(i) *Occurrence.*—The legume bacteria, as the name would suggest are generally present in soils in association with plants belonging to the natural order Leguminosae [Edwards and Barlow, 1909]. There are, however, a few plants in this natural order which are not infected by the bacteria [Leonard, 1925]. Occasionally the bacteria are also found in the roots of non-leguminous plants such as the Russian olive tree, *Clanorhus americanus*, and *casurina* [Snyder, 1925 ; Blake, 1932].

(ii) *Nomenclature*.—The nodule bacteria are referred to by different names according to the particular system of classification adopted. Bergey [1930] places all the known nodule bacteria under the genus *Rhizobium* and six species have been recognized in this group, viz., *Rh. leguminosarum* Frank, *Rh. trifolii*, *Rh. phaseoli*, *Rh. meliloti*, *Rh. radicicola* and *Rh. japonicum*.

(iii) *Morphology and life-cycle*.—The general characteristics of the above group of organisms have been defined by Bergey [1930] as 'minute rods, motile when young, branching rods abundant and characteristic when grown under suitable conditions, obligate aerobes capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates and in the absence of organic nitrogen compounds, produce nodules in the roots of leguminous plants'.

Whether grown in culture media or soil, the bacteria exhibit a clear and definite life-cycle. According to Bewley and Hutchinson [1920] the life-cycle consists of five stages: (a) the small non-motile pre-swarmer coccus, (b) the larger non-motile coccus, (c) motile swarmer, (d) rod form and (e) the stage of high vacuolation (bacteriod). In the nodule tissue cocci, small evenly staining rods and banded granular rods usually occur at successive age levels in the growing nodule. In the nodule, the granular rod stage consists of swollen, pear-shaped and banded cells which are called bacteriods. Striking deviations in the life-cycle have been described by Gibson [1928], but more work is needed before the less common cell types can be established as normal components of the life-history. Gangulee [1926] has reported that in culture media the various stages are found to occur simultaneously but in varying proportions. The distinct life-cycle has a bearing on the spread of bacteria through the soil and consequently on the infection of the host plant [Thornton and Gangulee, 1926]. It helps in the movement of the organism. The amount is not detectable until the majority of the organisms have passed into the coccus stage and is thus presumably due to the active migration of flagellated swimmers [Thornton, 1936,3]. Banded rods are the most common form met with in the life-cycle of the groundnut-nodule organism [Rajagopalan, 1938].

Lewis [1938] has studied the cell inclusions of *Rhizotia* and has concluded that the life-history of the organism is not cyclogenic in the sense that special reproductive cells, gonidia or spores are formed in the process of reproduction.

(iv) *Cultivation and cultural characteristics*.—These organisms can be grown in culture media having the following composition [Wieland, 1922]:—Mannitol 10 gm., NaCl 0.2 gm.,  $K_2HPO_4$  0.5 gm.  $MgSO_4 \cdot 7H_2O$  0.2 gm.,  $CaSO_4 \cdot 2H_2O$  0.1 gm.,  $CaCO_3$  1 gm. and yeast water 100 c.c. in a litre of medium. Suitable modifications of this medium have been made by different workers for isolation and cultivation of this organism [Edwards and Barlow, 1909; Carrol, 1934, 2; Whiting, 1915].

Attempts have been made to study the cultural characteristics of the different strains of organisms present in various species of plants by growing them in artificial media. Thus Stevens [1925, 1] has found that the 13 strains of alfalfa and sweet clover studied by him are divisible into two groups based on their cross-agglutination test and nitrogen-fixing power in sand cultures. He [1925, 2] has also observed that litmus milk is more useful than janus green or cresol purple in bringing out the characteristics of these groups. From the physiological reactions Wright [1925] concludes that these do not

represent two distinct species but two biotypes each of which varies around a mean of its own. Organisms of the groundnut nodule are also divisible into two physiological groups [Rajagopalan, 1938]. Madhok [1935] has studied the cultural characteristics of the organism causing nodules on the roots of berseem (Egyptian clover).

When grown in sugar media, organisms of alfalfa, clover, pea and dales produce an acid reaction, while those of soyabean, cowpea and lupine produce an alkaline reaction [Baldwin and Fred, 1927 ; Bushnell and Sarles, 1937]. Most of the wild legumes produce an alkaline reaction in sugar media [Conklin, 1936]. Palacios and Bari [1936] have reported that Indian nodule organism isolated from *Cajanus indicus* produces both alkaline and acid reactions depending on the nature of sugars in the medium.

Classification based on fermentation characteristics of the organisms is in harmony with flagellation, or other cultural and serological reactions [Baldwin and Fred, 1927 ; Clarke and Hanson, 1933 ; Carrol, 1934, 1]. Anderson and Walker [1932] have suggested that viscosity in solution cultures may also be of value in differentiating *Rhizobium* cultures.

The H-ion concentration of the medium exerts considerable influence on the morphological characters and growth of the organism, thus *Rh. radicicola* isolated from kidney bean, red clover, cultivated pea and hairy vetch do not grow in media above pH 7.0 or below pH 4.5 [Smezok, 1938].

Among the serological reactions of this organism, the complement fixation test and agglutination test are of equal value in identifying the different strains. It is of interest to note in this connection that a close protein kinship exists among the strains isolated from 15 species of *Crotolaria* [Carrol, 1934].

Burke and Burkey [1925] have found that by growing *Rhizobium radicicola* in increasing concentrations of dye, the organisms are modified to tolerate 1 in 1000 of genitain violet.

West and Wilson [1938, 1, 2] have shown that vitamin B<sub>1</sub>, an active heat stable substance and riboflavin are synthesized by growing cultures of *Rh. trifolii*.

#### *Relationship between leguminous plant and nodule bacteria*

(i) *Infection of the host plant*.—Simultaneously with the unfolding of the first true leaf, the root secretes a substance, probably of the nature of coenzyme R, and at this stage the first appearance of the nodule on the seedling takes place. The nature of the secretion as also the seat of its formation are not known. The removal of the leaf does not delay nodule formation [Thornton, 1929], probably the coincidence is incidental and the unfolding of the leaf may not have any direct bearing on nodule appearance.

Thornton [1936, 1] has worked out the histological changes that take place in the root tissue during infection. The nodule bacteria secrete a thermostable, filterable active substance which comes in contact with the root tip and produces a characteristic curling [Thornton and Nicol, 1936]. This curling of the root tip is a necessary prelude to infection [McCoy, 1932]. In the early stages a small colony of bacteria appears close to the apex of the root. This induces irregular growth of the root hair, so that the hair curls over to form a tight spiral. As a result of this, a local weakening of the cell-wall takes place

and the bacteria enter the root at this point. In liquid cultures *Rhizobia* induces hyperplastic effect on the roots of peas; it also causes hypertrophy with the deformity of cortical cells and is dependent on the elongation of root cells [Mollard, 1913].

There is a marked specificity in the infection of host plant by bacteria. Eighteen host specific groups have so far been recognized. Infection of the host plant outside the host specific groups have been described [Allen and Allen, 1939], but it is of very rare occurrence. The serological reactions of the nodule organism have been correlated with host specificity [Baldwin and Fred, 1927] and ability for cross inoculation also corresponds with the serological and other biochemical reactions of the bacteria [Walker, 1928]. There is no correlation between the amount of indol-acetic acid produced in synthetic media in presence of tryptophane by various strains of *Rhizobia* and their ability to induce formation of nodules on the roots of leguminous plants [Georgi and Bond, 1939]. The immunity is not connected with the preliminary curling of the root hair—probably it is a protein reaction.

(ii) *Nodule formation*.—When the bacteria have penetrated into the root hair they form a thread-like growth of zooglea, known as the infection thread, which passes down the hair and penetrates the cortex of the root. This causes the root cortex to become meristematic and by division to produce the young nodules. The cell division may extend inwards to involve the endodermis or even the pericycle cells. This might have an important bearing on the diffusion of nutrients into the nodules. Cell division extends beyond the cells that are actually infected and is perhaps due to the secretion of some stimulant by the bacteria.

The bacteria are distributed through the cells of the young nodules in three different ways in different legumes [Milovidov, 1928]. In the first type, common to most legumes, the bacteria spread by means of infection threads which pass through the perforations in the cell wall. Thornton [1930, 2] has observed that secondary release of bacteria may take place in this type of distribution by the formation and subsequent breaking up of blisters formed from the infection thread. In the second type which occurs in *Serradella*, the bacteria infect the intercellular spaces and spread by that means. The third type is met with in lupins, in which the bacteria invade the dividing host cells in the young nodules and are thus distributed in the daughter cells.

Ultimately the presence of bacteria in the host cells stops their division while permitting them to increase in size. The combined activity of uninfected cells causes further growth of nodules, and this seems to be essential for the healthy functioning of the nodule. The presence of the nodule in the cortex induces an outgrowth of vascular strands from the stele. A secondary endodermis is formed surrounding each vascular strand and enclosing the central infected tissue as far distally as the meristem cap. The cytoplasm of the infected cells in the centre of the nodule becomes closely packed with bacteria which later on become branched and constitute the so-called 'bacteriods', and a group of these bacteriods form the nodule [Thornton, 1936, 3]. Aeration helps the formation of the nodules, while passing nitrogen completely prevents nodulation [Virtanen and Hausen, 1936]. Wilson *et al.* [1933] have studied the effects of supplying additional CO<sub>2</sub> to clover and alfalfa on their nodule formation.

(iii) *Symbiosis between the plant and the bacteria*.—Evidence has been brought forward by a number of workers [Wilson, *et al.*, 1932 ; Barthel, 1921, 1926 ; Hopkins, 1929 ; Allison, 1929 ; Lohnis, 1930 ; Galestin, 1933 ; Pietz, 1937 ; Virtanen and Hausen, 1935, 2] that the host plant plays a more subtle role than mere furnishing room and board for the bacteria. It has been proved by these workers that the bacteria cannot fix nitrogen outside the host plant. Fred [1909] claims that certain amount of nitrogen is fixed by the bacteria even without the host plant, but this requires further confirmation. That the process of nitrogen fixation is the result of symbiotic relationship is further confirmed by the fact that the host plant by itself cannot fix nitrogen without the aid of the bacteria [Skallow, 1936 ; Guitschanoff, 1935]. The efficiency of nitrogen fixed is dependent more on the location than on the size or the number of the nodules [Rajagopalan, 1938].

The life of the bacteria in the host tissue has a definite bearing on its activity and efficiency of nitrogen fixation. A number of workers [Wumshik, 1925 ; Albrecht, 1920 ; Kalnius, 1938] have shown that the passage of the bacteria through the host plant results in increased activity. The beneficial effect on the host plant is the resultant of two factors, nitrogen fixation and inhibitory effect on plant growth due to growth of bacteria in the nodule. The effectiveness of the organism and its ability to aid the host plant are unrelated factors [Allen and Baldwin, 1931].

Erdman [1929] has reported that there is rapid translocation of the nitrogen fixed by the bacteria from the nodules to the seeds of the plant. Bond [1933] has reported that there is a quantitative relationship in the transfer of nitrogen between the bacteria and the host cells. He [1936] has further shown that more than 80 per cent of the nitrogen fixed by the nodule bacteria is liberated without appreciable delay in the host cytoplasm. The mechanism seems to be one of passive excretion by the bacteria in which the nitrogenous substances represent a phase of bacterial respiration rather than part of the process of bacterial synthesis.

Pietz [1937] has recorded the presence of the oxidation product of dihydroxy phenyl-alanine in the roots. This substance which changes in colour at specific  $\eta$  values is highly significant for its behaviour in the roots ; this to a large extent determines the growth of the bacterium. The dopa action alone does not, however, explain its role in the symbiosis mechanism.

#### *Factors determining the host-bacteria equilibrium*

The proper functioning of the bacteria within the host depends upon the maintenance of a physiological equilibrium between the host and the bacteria. Thornton and Rudolf [1936] have shown that the presence of  $\text{NO}_3$  checks the infection of root hairs by protecting them against the normal action of bacterial secretions deforming them. This appears to be connected with the C : N balance in the root hairs. Secondly the cell walls of the distal meristematic cap cease to divide and form an increasingly thickened wall and thus isolate the bacterial tissue which later on shows symptoms of starvation finally becoming necrotic [Thornton, 1930, 1]. The equilibrium breaks down in old nodules even without the presence of  $\text{NO}_3$ . This is due to the bacteria actually attacking the host tissue thereby becoming parasitic. Brenchley and

Thornton [1925] have shown that this state may be induced even in young nodules by certain changes in food supply, such as boron deficiency and failure of carbohydrate supply. It is probable that the change to parasitism is due to the bacteria being cut off from the supply of carbohydrate owing to the failure of the vascular strands.

(i) *Carbohydrates*.—An adequate and unhindered supply of carbohydrate seems to be essential for the healthy functioning of the nodule in the host tissue. Probably this supplies the energy for the fixation process [Ruffer, 1932]. Rippel and Krause [1934] have found that there is relation between carbohydrate and nodulation. Allison [1934] has observed that the nodules are located in the upper part of the root nearest the carbohydrate supply and when the supply becomes deficient they become dormant and in other cases they attack the host tissue to obtain food. In general, increased  $p\text{CO}_2$  (partial pressure of  $\text{CO}_2$ ) augments photosynthesis which in turn nodulation and nitrogen fixation. The effect is most pronounced between 0.03 and 0.01  $\text{CO}_2$  and is due to increased partial pressure and not the total amount present [Vita and Sandrinelli, 1935]. In the case of red clover, Georgi *et al.* [1933] have observed that higher  $p\text{CO}_2$  is more effective. Allam [1931] has shown that light influences symbiosis between legume bacteria and host plant. Allison [1934, 1935] has suggested that high carbohydrate content is not essential for bacterial entrance but is necessary for root growth and that the bulk of the carbohydrate supply is used in growth and respiration of host tissue.

Certain amount of work has been carried out with a view to finding out the relative efficiencies of the different sugars as a source of supplying energy to *Rhizobium* cultures. The growth of *Rh. leguminosarum* in different sugars was in the following order: sucrose, lactose, glucose and dextrin [Madhok, 1935]. In agar media Reynolds and Werkman [1935] have found that sucrose was better than mannitol for the growth and longevity of *Rhizobia*. Georgi *et al.* [1933] have reported that addition of mannitol is not useful for nitrogen fixation; concentrations above 0.25 — 0.50 is detrimental, when inoculated with non-homologous species of bacteria and the plants die of nitrogen starvation even though carbohydrate is applied to them. No significant difference is observed in *Rh. meliloti* in the rate and extent of oxygen consumption in the media containing glucose, mannitol or sucrose. Arabinose was distinctly superior to other carbohydrates as a source of energy to *Rh. japonicum*.

Wilson [1935] and Walker and Anderson [1934] have shown that it is the carbohydrate-nitrogen relationship obtaining in the inoculated plants that is more important in symbiotic nitrogen fixation. Allison [1934] has found that the addition of nitrate alters the C : N ratio so that the carbohydrate is all used up for top growth and thus it inhibits nodulation. Honl [1930] has observed that the C : N relationship of the legume remains constant at various stages of growth while in non-legumes it varies.

More recently Allison and Ludwig [1938] have made a critical study of the available data in regard to legume nodule development in relation to available energy supplied and have concluded that the carbohydrate supply hypothesis accounts for the varying degrees of nodulation under different growth conditions, more adequately than the subsequently proposed carbohydrate-nitrogen hypothesis.

Weniger [1923] has suggested that the eventual energy requirement of nodule bacteria is so small as to be insignificant, the energy being hardly sufficient to support the life of bacteria—the requirements of non-symbiotic bacteria were five times as great. He has further suggested that the organisms are so constituted as to be able to transform energy incident to an exothermic nitrogen fixation process. Rippel and Poschenreider [1928] have found that soybean bacteria used enormous amounts of energy for nitrogen fixation, 104 calories in one experiment and 50 calories in another. Neal and Walker [1936] and Walker [1934] have shown that only one-third of the carbohydrate is oxidized to  $\text{CO}_2$  and water and used for energy. It would appear that the carbohydrate is needed not only to feed the bacteria, but also the meristem cap of the host plant leading to the continual formation of young nodule tissue.

(ii) *Minerals and humic acid.*—Allison and Hoover [1935, 1] have shown that natural humic acid stimulates growth and oxygen consumption by *Rhizobia*; thus over a range of 0 to 600 p.p.m. of dry matter it was proportional to the quantity used. Small amounts of iron salts stimulated growth of *Rhizobia*; chloride was better than sulphate. The optimum concentration of iron was found to be 10 p.p.m. Smith [1907] has studied the effect of various iron salts in concentrations ranging from 0.00005 to 0.05 on *Asteralagus sinicus*. Stimulation was greatest with ferric malate and chloride, less with ferric salts (sulphates, citrates, oxalate and tartrate) and zero with other salts. Depression was greater with  $\text{FeI}_2$  and  $\text{Fe}(\text{NO}_3)_2$ , less with  $\text{FeCO}_3$  and least with ferric malate. Calcium adsorbed on colloidal clay transforms the abnormal forms into normal and effective nodules; the reason for the special activity of the adsorbed calcium is not understood. Ba in place of Ca produces the opposite effect [Albrecht *et al.*, 1937]. Itano and Matsuura [1936, 2] have recorded that titanium salts have specific morphological influence on the bacteria. Graham [1938] has studied magnesium as a factor in nitrogen fixation by soybeans and has shown that increase of Mg made it possible for the plant to make a more efficient use of Ca offered at a given level. The supply of exchangeable bases seem to be a limiting factor in legume growth and nitrogen fixation.

(iii) *H-ion concentration.*—The optimum pH for the growth of nodule bacteria in nutrient gelatin lies between 6.5 and 7.5 [Virtanen *et al.*, 1931, 1]. Maximum respiration and growth takes place near neutrality with constant activity between pH 6 and 7.8 [Thorne and Walker, 1935, 2]. In *Rhizobium meliloti* and *Japonicum* the optimum reaction for respiration is more alkaline than the optimum for growth [Thorne and Walker, 1936, 1]. The effect of soil acidity on nodule formation is not due to its effect on growth of roots of the plant but due to its effect on bacteria when it exists in the soil non-symbiotically [Karrakar, 1927].

(iv) *Accessory growth factors.*—Plant extracts greatly influence the growth of nodule bacteria [Allison, 1927]. Yeast and cane sugar contain the necessary growth factor for *Rhizobia* which can be extracted with absolute alcohol; no other substance tried was able to replace the factor present in yeast extract [Thorne and Walker, 1935, 1]. Potato extract stimulates the growth of *Rhizobia*, and asparagine to a less extent [Sarles and Reid, 1935]. Nilsson [1938] has shown that addition of materials like leguminous plants, molasses or

yeast is required for growth of *Bacillus radicolica* in synthetic medium. Verner *et al.* [1936] have reported that reproduction and nitrogen fixation in nodule bacteria are proportional to concentration of *bios* in the medium and that in its presence the nodule bacteria can bind molecular nitrogen outside the host plant. Accessory substances present in bean nodule and yeast extract are soluble in water and alcohol and chloroform and are non-dialysable [Itano and Matsuura, 1936, 1]. Sauerkraut has a more effective growth factor than yeast [Albrecht *et al.*, 1937].

The factor present in sauerkraut is soluble in alcohol and dilute acetic acid, slightly soluble in methyl alcohol and insoluble in petroleum ether and pyridine. It is not absorbed on Fuller's earth and passes through colloidal membrane but is destroyed by electrodialysis with complete chloride removal [Albrecht *et al.*, 1937]. On electrodialysis of bean nodule most of the accessory substances were found in the cathodic chamber. There was no relation between accessory substances and nitrogen fixation [Itano and Matsuura, 1937, 1938]. The growth-promoting substance is an organic complex of relatively low molecular weight. It is probably not rhizopin auxin or inositol and in some respects it resembles *bios* [Clarke, 1936].

The important function of accessory growth substances of *Rhizobia* seems to be the provision of an initial H-donor which in turn lowers  $r_h$  and supplies a readily available initial source of energy. [Virtanen and Laine, 1936; Thorne and Walker, 1936, 2].

Recently, Laird and West [1938] have found that certain components of 'Wildiers' complex are capable of replacing the stimulatory action of yeast extract on strains of *Rh. trifoli* and this was proportional to the increased urease activity produced by this factor. A number of compounds tried including vitamin B<sub>1</sub> could not bring about this action. Nilsson *et al.* [1938] have shown that a second factor obtained by acid extraction from yeast in crystalline form is, in conjunction with vitamin B<sub>1</sub>, highly active in promoting the growth of *Bacterium radicolica*; the vitamin also causes increase in size of the bacterium and seems to favour formation of bacteroid branching. Steinberg [1938] has suggested that a second accessory factor is also necessary for the growth of *Rhizobia* and for which the name *Rhizobiosin* is proposed. West and Wilson [1939] have adduced evidence to show that the effect is due at least in part to the presence of thiamin and flavin in those products. Allison and Minor [1938] have reported that in the case of 19 strains of *Rhizobia* tested, the addition of coenzyme R to the medium is necessary in order to make appreciable growth; the need for this was equally evident when the organism was growing in combined nitrogen, thereby showing that it may not have any part to play in the fixation of nitrogen. The factor is of organic nature although its chemical nature has not yet been determined. More recently, Bjälfve *et al.* [1938] have shown that the growth effect of vitamin B<sub>1</sub> on *Rhizobium* holds good only for half of the strains isolated from clover and it does not hold good for those found in peas, lupines and beans.

Allyn and Baldwin [1930] have shown that the legumes are very sensitive to slight changes in the oxidation-reduction character of the medium and it is likely that the beneficial effects of certain extracts may be due, in some instances, to their effect on this potential. Thus, ordinary mineral-sugar media are too oxidized for optimum growth; a fair growth may take place even

in such unfavourable media if comparatively heavy inoculations are made, but the oxidation-reduction potential must be adjusted very accurately in order, for single cells, to initiate growth.

(v) *Alkaloids*.—The formation of bacterioids in *Bacillus radicola* is connected with the occurrence of plant bases, thus in the presence of caffeine it was possible to cause bacterioid formation in sterilized soils. Barthel [1926] has reported that formation of bacterioids in the nodosities is dependent on the presence of alkaloids in the roots. There is a relationship between the nitrogen utilized by the legume and the bases absorbed by it at different stages of growth [Stock and Rippel, 1929]. Caffeine influences the transformation of nodules into bacterioids but does not increase their capacity to fix nitrogen; the bacterioids probably cannot fix nitrogen of the air [Bazarewski, 1929; Borthelot, 1885]. In liquid cultures, caffeine in doses of 0.05–0.50 is an excellent stimulant, from 1.1 to 1.4 it is depressant, while above 1.5 it is toxic. Quinine and strychnine are more vigorous as stimulants between 0.05 and 0.10 and poisonous above 0.25. In solid cultures they are less vigorous [Messadrol et al., 1935]. Itano and Matsuura [1936, 1; 1937, 1938] have reported that alkaloids did not stimulate nodule bacteria, especially growth could not be observed with quinoline, and caffeine was most notable in producing large bacterioids which were associated with poor growth.

(vi) *Bacteriophage*.—Gerretsa et al. [1923, 1924] and Hitchner [1930] have reported that the bacteriophage isolated from nodes of leguminous roots is probably active in dissolving bacteria. The lytic action is specific. The bacteriophage stands 55–65°C., passes through colloidion membrane and is eight times more resistant to ultra-violet light than the bacteria. Grijns [1927] has found that the clover plant does not produce a bacteriophage and that under these conditions the presence or absence of the bacteriophage does not affect the growth of the plant. Demelon and Dumez [1938] have shown that the addition of bacteriophage filtrates, isolated from roots and nodules and heated to destroy the lytic agent, stimulated alfalfa growth.

(vii) *Nitrogen compounds*.—*Rh. meliloti* and *Rh. japonicum* produce ammonia from a number of amino acids tested;  $\text{NO}_3$  is reduced to  $\text{NO}_2$  and utilized by both species. There is a difference in the chemical action of various species on different nitrogen sources [Pohlman, 1931]. Amino acids are used directly by *Rhizobium* cultures and not through  $\text{NH}_3$  stage, since the pH of the medium remained constant and  $\text{NH}_3$  could not be detected [Virtanen et al., 1931, 2]. The decrease of nodulation in presence of soil nitrogen salts is due to an inadequate supply of carbohydrate in the roots; the bacteria themselves play only a secondary role [Allison and Ludwig, 1934]. The influence of combined nitrogen on symbiotic nitrogen fixation depends on how it alters the C : N relationship in plants [Wilson and Wagner, 1937]. Thus the addition of  $\text{NO}_3$  alters C : N ratio, so that the carbohydrate is all used up for top growth [Allison, 1934]. In *Rh. japonicum*,  $\text{NO}_3$ -nitrogen was better than  $\text{NH}_3$ -nitrogen [Neal and Walker, 1935]. Soluble nitrogen compounds which the plants utilize are not fixed by the bacteria [Georgi, et al., 1933]. The growth of the nodules is limited by the limiting amounts of nitrogen fixed, the coefficient number of nodules and percentage of nitrogen being  $0.57 \pm 0.10$  [Fred and Wilson, 1934].

Small amounts of glycine (0.1–0.5) result in partial or complete loss of infective ability of *Rhizobia* and *Phytomonas tumefaciens*, the loss being

complete after ten generations and permanent after thirty generations. Alanine, glycylglycine and dicynamide induce similar responses in several strains of *Phytomonas tumefaciens* [Longley *et al.*, 1937].

### *Products of metabolism of nodule bacteria*

(i) *Slime*.—Slime is produced by the bacteria only when they fix nitrogen. It is produced in faintly alkaline and neutral media but not in acid media. Lipine produces slime in neutral media which rapidly become acid. The best medium for the production of slime contains 0.06 per cent asparagine and 0.01—0.2 per cent of alkali phosphate [Smith, 1907]. Hopkins *et al.* [1930] have observed that addition of nitrates to the medium increases gum production. Gum production is a normal process in the metabolism of the organism; when purified gum is supplied to *Rhizobium*, it is not utilized as a source of carbohydrate [Anderson, 1933]. *Rh. radicicola* produces gum from different carbonaceous materials, such as mono and disaccharides, dextrin, inulin, leaven, polyhydric alcohols containing 3, 5 and 6 carbon atoms and sodium salts of lactic, malonic and succinic acids. It produces no gum from salts of fatty acids, succinimide, malonamide and amino acids. The synthesis of gum is completely inhibited by high concentrations (5-10 per cent), optimum being 1-2 per cent. It is a polysaccharide containing glucuronic acid [Cooper and Peterson, 1937]. The gum produced by cross inoculation groups is precipitated by acetone and is free from nitrogen. The carbon content varies from 36.4 to 40.6 per cent; on hydrolysis it yields glucose and not pentoses; it contains 4.1—25.3 per cent uronic acid and the complex is probably glucuronic acid [Greaves and Anderson, 1914]. Condition for the production of gum is similar to that of *Azotobacter* [Cooper and Peterson, 1937]. More recently, Cooper *et al.* [1938] have shown that there is a close similarity in the polysaccharides of *Rh. radicicola* and those of *Azotobacter* and have suggested that these belong to the same class as the polysaccharides of the pneumococcus types II and III.

Georgi and Wilson [1933] have suggested that the gum may be an intermediate step in the oxidation of carbohydrate into  $\text{CO}_2$ . The evidence for this view is that if the organism is grown in the presence of a limited quantity of oxygen, so that respiration is arrested after a few days' growth, only 50 per cent of the glucose which disappears could be accounted for as  $\text{CO}_2$ , whereas in presence of sufficient oxygen in the medium the amount of glucose carbon which disappeared as  $\text{CO}_2$  rose to 70-80 per cent.

(ii) *Proteins*.—The properties of protein of legumes being very similar to those of casein, Rukuzin and Pekrskaya [1920] have suggested the name vegetable casein for it. From a study of the protein make-up of roots and tubercles of *Vicia faba*, it is concluded that the co-presence of free amino acids and of reducing substances in considerable quantity in the bacterial tissue of tubercles indicates the existence of a direct relationship between these substances and the synthesis of proteins [Parisi *et al.*, 1926]. The chemical composition of the nodular tissue is not different from that of the other part of the plant. The dry matter of nodule bacteria consists chiefly of carbohydrates; it contains 52.8—54.6 per cent carbon, 4.4—4.9 per cent of nitrogen, 11.4—22.6 per cent fat [Carrol, 1934, 1]. 20 per cent of the nitrogen of the nodules is arginine.

The soluble portion of fresh nodular tissue contains much arginine and is probably the basic amino N previously thought to be of importance in symbiotic nitrogen fixation. There is no difference in the composition between different species. The total nitrogen in heavy gum producers is 2 per cent, while that of alfalfa is 8.4 per cent [Umbreit and Burris, 1938]. The nitrogen content of bacterial tissue increases with the age of the culture and with decrease in C : N ratio [Rajagopalan, 1938].

(iii) *Fermentation products*.—Acids are produced by fermentation of sugars with alfalfa bacteria. 5.5 per cent of the weight of sugar was fermented into acids with sucrose, and 7.3 per cent with lactose. Anderson *et al.* [1928] have detected pyruvic acid in *Rhizobium* culture. The power to ferment sugars by *Astragalus sinicus* (Genge) varied in the order arabinose, xylose, glucose, galactose, mannose, fructose, sucrose, mannitol, lactose, maltose, raffinose and dextrin. Nitrogen source is not necessary for growth, but is necessary for fermentation [Matsuura, 1935]. During fermentation of sugars by groundnut nodule organism acetic acid, ethyl alcohol, traces of aldehyde, tartaric acid and carbon dioxide were detected. In nitrate medium 14 per cent of the carbon supplied is used for cell formation, 21 per cent is oxidized to  $\text{CO}_2$  and the rest converted into other by-products [Rajagopalan, 1938]. The fermentation is of the pyruvic acid type [Virtanen *et al.* 1933, 2]. McBurney *et al.* [1935] have suggested that pantothenic acid produced by *Rh. meliloti* plays a part in the carbohydrate anabolism of the plant.

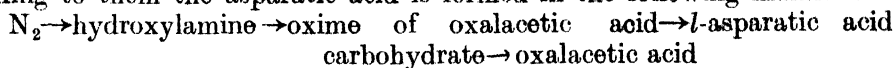
#### *Biochemical mechanism of symbiotic nitrogen fixation*

(i) *The first intermediate product in symbiotic nitrogen fixation*.—With a view to finding out the first chemical step in fixation of nitrogen in the symbiotic system, several workers have attempted to demonstrate the first intermediate product formed during the process. Notable among these workers are Winogradsky, Virtanen and his associates, and Orcutt.

According to Winogradsky [1933, 1938] and Winogradsky & Winogradsky [1936] the liberation of ammonia from root nodules is the result of nitrogen fixation and not ammonification because (a) maximum production of  $\text{NH}_3$  takes place on the second day followed by a decrease, (b) the effectiveness of antiseptics and anaesthetics, (c) evolution of ammonia from dried and powdered nodules at 40—50°C., and (d) the fact that neither the roots of maize nor legume free from nodules were capable of forming ammonia. Since Winogradsky failed to demonstrate that the nodules in his experiments were fixing nitrogen and since all previous experiments with detached nodules under conditions similar to those used by him, have been negative with respect to fixation, his conclusion that ammonia is the first product is hardly acceptable.

Virtanen and his colleagues [1936] have brought forward evidence to show that asparatic acid, which is excreted along with lysine, is the primary product of nitrogen fixation. Virtanen and Laine [1936; Virtanen, 1936, 2; Virtanen and Laine, 1938, 1] have detected small amounts of  $\text{NO}_3$  and  $\text{NH}_2\text{OH}$  in addition to asparatic acid; they have suggested that asparatic acid may be formed from hydroxylamine and oxalacetic acid and the  $\text{NO}_3$  may arise from oximes originally present in the cultures. They [1937] have further shown that the root nodule bacteria split off  $\text{CO}_2$  from asparatic acid and thus produce alanine.

By *in vitro* experiments it has been possible to demonstrate that when pyruvic acid and l-asparatic acid are added to crushed pea plants alanine is formed. This transfer of  $\text{NH}_2$  from asparatic acid to keto acid is similar to that occurring in animal tissues. They have further suggested that the nitrogen is probably converted by an unknown intermediate into hydroxylamine which probably reacts with oxalacetic acid to form oxime which is later reduced to give l-asparatic acid [Virtanen, 1938]. Virtanen [1937, 2] has demonstrated that the legume bacteria split off one of the carboxyl groups from l-asparatic acid forming  $\beta$ -alanine. The reaction is almost quantitative at pH 7.0 and is accomplished by living bacteria. In support of this, they have also demonstrated nitrogen fixation by excised root nodules in oxalacetic acid medium. According to them the asparatic acid is formed in the following manner :



More recently, Virtanen *et al.* [1938] have adduced evidence to show that the legume bacteria contain two different amino acid carboxylases, asparatic decarboxylase and glutamic decarboxylase. Wilson [1939, 2] has, however, failed to confirm Virtanen's findings; he was unable to accomplish nitrogen fixation by excised root nodules; he has also failed to detect the presence of oxalacetic acid in the medium by the aniline manometric method of Ostern.

From a study of nitrogen compounds in different parts of the legumes supplied with free and fixed nitrogen, Orcutt [1937] has suggested that the only fraction that appears to offer possible significance in the fixation process is the basic amino fraction. Umbreit and Burris [1938] have confirmed this and have further shown that this fraction has an unusually high content of a substance which gave ammonia on hydrolysis with alkali. This has been tentatively identified as arginine. The conclusion drawn from these composition studies can, however, be regarded only as suggestive.

It is evident from the foregoing that our present knowledge of the first chemical step in symbiotic nitrogen fixation is still meagre for want of exact and reproducible data.

(ii) *Excretion of nitrogen compounds from root nodules.*—The nitrogen compounds excreted by nodules are mainly amino acids; no  $\text{NO}_2$  or  $\text{NH}_3$  is present, but small amounts of amides and volatile bases, probably amines, are found in the dialysed portion [Virtanen *et al.*, 1933, 1]. 87—98 per cent of the total nitrogen excreted is amino nitrogen; asparatic acid accounts for 50 per cent while the other half can be precipitated by phosphotungstic acid, but is not arginine, cystine, histidine or any aromatic amino acids [Virtanen and Laine, 1935]; probably it is lysine [Virtanen *et al.*, 1936]. The major part of the amino acid excreted from quartz cultures of inoculated peas before flowering is l-asparatic acid nitrogen, while if the peas are almost mature, chiefly alanine is found in the cultures [Virtanen and Laine, 1937]. Nitrogen excretion from inoculated plants takes place from the bacteria inside the nodules and not from the roots [Virtanen *et al.*, 1937].

Air supply results in an increased rate of nitrogen excretion [Virtanen and Hausen, 1934, 1935, 1]. Excretion of nitrogen is highest in young roots [Virtanen and Hausen, 1935, 2] and the rate of excretion is maximum before blooming [Virtanen *et al.*, 1936]. The nitrogen compounds, especially

asparatic acid and lysine, are excreted only slightly in water, more so if there was sand or clay in the water and still more so in soils in which non-legumes are growing [Virtanen, 1936, 1]. Virtanen and Hausen [1936] have shown Hausen, that direct contact of the roots and the nodules with solid particles is necessary for nitrogen excretion.

Virtanen *et al.* [1937] have shown that non-legumes in association with legumes facilitate excretion of nitrogen. In sterile cultures of lupines amino nitrogen was excreted in considerable quantity when the plant was young [Isakova and Andreev, 1938]. Under favourable conditions of photosynthesis there is no or poor excretion of nitrogen. Lowering of temperature and shading cause more excretion. Ammonia, nitrate and cyanamide lowered nitrogen excretion in white clover [Wilson and Wyss, 1937]. The extent of nitrogen excretion depends largely on the strain of the organism and the quantity of medium available [Virtanen *et al.*, 1937].

The legumes receive their nitrogen supply from the nodules in the form of amino acids which are the products of nitrogen fixation and not protein break-down [Virtanen *et al.*, 1937]. Virtanen *et al.* [1933, 1] have shown that asparatic acid is an excellent source for leguminous plants but is entirely unsuitable for cereal plants. The excreted nitrogen is utilized by non-legumes, oats, beets, and wheat used 50 per cent while potatoes 90 per cent; with increase in excretion, peas utilize only a fraction of the nitrogen fixed by the nodules [Virtanen *et al.*, 1937]. The excreted nitrogen is also utilized by barley and other plants grown by the side of the legumes; barley utilizes lysine in preference to asparatic acid [Virtanen *et al.*, 1936].

Excretion of nitrogen is not universally obtained under experimental conditions which are apparently identical [Wilson, 1937]. Thus Bond [1937, 1938] has reported that no nitrogen was excreted in sand cultures by soyabean and broad bean, and small amounts only with peas. Perhaps the failure to detect excretion is due to use of coarse sand which lacks absorptive capacity [Virtanen, 1937, 1]. By reducing the exposure to sunlight of pea plants previously exposed to normal daylight, Strong and Thrumble [1939] have confirmed excretion of nitrogen by roots. Recently, Sharper [1939] has described a simple technique by which he has detected excretion of nitrogen in lucerne plants inoculated with *Rh. meliloti*.

The origin of the excreted nitrogen is not, however, very clear. Virtanen and Hausen [1935, 2] have reported that it is not due to mechanical injury since it takes place even in agar cultures. It represents the primary product of nitrogen fixation and not decomposition products of protein, since no excretion takes place in uninoculated plants [Virtanen *et al.*, 1936]. On the other hand, Wilson and Burton [1938] have reported that excretion does not always accompany fixation and it appears to be the exception rather than the rule in greenhouse studies.

(iii) *Respiration studies.*—Barthel [1932] has carried out experiments with different strains of *Bact. radicicola* in culture solution under reduced oxygen pressures and has shown that by an amount of only 0.5 per cent of oxygen in a mixture of nitrogen and oxygen, or of hydrogen and oxygen, the growth of the bacteria amounted to about 25 per cent of the growth in the checks held under normal aeration.

The rate of respiration for nodule bacteria is considerably higher than that of other bacteria. From a study of its respiration using various metabolites, Georgi and Wilson [1933] have classified the organisms as obligate aerobes. The organism grows well even under low oxygen tensions (less than 0.03 atm.). In low oxygen tensions glycolysis rather than butyric fermentation takes place [Virtanen *et al.*, 1934]. During the dissimilation process the R. Q. (the respiratory quotient) reaches 1.18 after which it decreases to 0.8 and remains constant, probably this marks the change from carbohydrate to protein metabolism and a large part of the oxygen thus not accounted for may be used for the formation of new cell tissue.

The respiratory quotient of nodule bacteria varies from 0.90 to 1.10 ; in the majority of bacteria it is slightly greater than 1.0 [Wilson and Peterson, 1933]. In glucose yeast extract medium the R. Q. was consistently less than 1.0 [0.85—0.96]. With  $\text{NaNO}_3$  in glucose the R. Q. increased to 1.07—1.17,  $\text{NO}_3$  being used as an H acceptor in place of  $\text{O}_2$ . In  $\text{NH}_4\text{Cl}$  the R. Q. was less than 1.0 [Anderson and Walker, 1933]. The R. Q. in *Rh. trifolii* is higher than in other species.

The utilization of carbon by *Rhizobia* decreases with increase in concentration of sugar ; with 1 per cent glucose all carbon was used up, while with 2 per cent only 50—70 per cent was used up [Wilson and Peterson, 1933]. The rate of oxygen consumption increases with increased oxygen pressure ; with decrease in oxygen concentration, consumption of oxygen was repressed in *trifolii* but not in *meliloti* and *lupini*. Growth of nodule bacteria in an atmosphere containing 0.5 per cent oxygen (in  $\text{H}_2$  or  $\text{N}_2$ ) was approximately 25 per cent that occurring under normal conditions [Anderson, 1933]. Respiration in *Rh. trifolii* is pronounced in an atmosphere in which  $\text{CO}_2$  is increased to 0.05 per cent [Konishi *et al.*, 1936].

A respiratory co-enzyme, which is essential primarily for respiration and indirectly for growth, is present in relatively high concentrations in yeast, cane molasses, humic acid and commercial egg albumin. Yeast extract increased the rate and extent of oxygen consumption in all strains of *Rhizobia* [Walker and Anderson, 1933]. Half the maximum growth is obtained with 16—20 p.p.m. of these extracts in the synthetic medium [Allison and Hoover, 1934]. Such stimulation could not be traced to the presence of nitrogen, carbohydrate, vitamins or salts in these extracts. Neither the nitrogen nor sugar requirements of *Rh. trifolii* are specific [Allison and Hoover, 1934]. Co-enzyme R. increases bacterial respiration two to five fold within an hour and the rate of growth of the organism is increased 20—30 times ; but it has no function in nitrogen fixation. Thorne and Walker [1934] have, however, suggested that the stimulation obtained by the above materials can be satisfactorily explained on the basis of their nutritional value and were unable to substantiate the assumption of a co-enzyme for respiration. The amount of oxygen consumed was greater in yeast extract medium than any other medium.

Wilson [1938] has studied different substrates as H-donators with oxygen and methylene blue in *Rhizobium* cultures. Among sugars, glucose and arabinose were the best. With polyhydric alcohols the cultures were active towards oxygen but not methylene blue (with the exception of sorbitol) ; probably in these oxidations an aldose is formed as an intermediate product. Among the organic acids the highest respiration occurred with fumarates and

succinates. From a study of the oxidation of different substrates in *Rh. trifolii* and *meliloti* cultures, Konishi and Kawamura [1938] have shown that different kinds of oxygenases are present in the cells of nodule bacteria. Catalase was more positive in bacterial culture from clover, alfalfa, gence, lupine and soybean. Peroxidase was more pronounced than catalase, especially in clover and soybean.

From a study of the physiological effects of various materials, such as yeast extract, Thorne and Walker [1936, 3, 4] have concluded that the role of the H-donor in this organism appears to be twofold; firstly, it tends to lower the oxidation-reduction potentials and secondly, it furnishes the organism with a rapidly available initial source of energy.

(iv) *Enzyme systems in symbiotic bacteria*.—The study of nitrogen-fixing enzyme system present in the symbiotic bacteria is difficult because of the two component nature (plant and bacteria) of the system responsible for nitrogen fixation. Nevertheless, a beginning has been made in this direction by Wilson and his colleagues. They have adopted the classical Warburg manometric method that is generally used for the study of enzyme system concerned with respiration of intact cells. By using this technique they could study the various factors responsible for nitrogen fixation in the symbiotic system. Rigorous statistical treatment of the mass of data obtained from carefully controlled experiments has been employed to determine the significance of their results.

It is well known that the rate of reaction in an enzyme system depends on the concentration of substrates. Using red clover plants Wilson [1936; 1939, 2] has studied the dependence of nitrogen-fixing reaction on the  $pN_2$  in the atmosphere. The maximum fixation was obtained when the  $pN_2$  reached 0.15—0.20 atmospheres, and there was a significant decrease in the quantity of nitrogen fixed only after the  $pN_2$  was reduced to 0.1 atmosphere. On the basis of available data the Michaelis constant,  $KN_2$  (the thermodynamic dissociation constant of the nitrogen-fixing reaction) for symbiotic nitrogen fixation appears to be  $0.05 \pm 0.005$  atmosphere.

It would be of interest to study the influence of  $pO_2$  on nitrogen fixation in view of the fact that molecular oxygen is involved in some of the mechanisms postulated in symbiotic fixation. From a study of the influence of oxygen under various pressures on the assimilation of nitrogen in the free and fixed state, Wilson and Fred [1937], Wilson and Bond [1936] and Thorne and Burris [1938] have shown that molecular oxygen does not play any direct role in the mechanism of nitrogen fixation. However, it may indirectly influence the rate of fixation through effects on the carbohydrate relationship in the host plant.

In the course of his studies on the influence of different nitrogen pressures Wilson found that the  $pN_2$  function (the relation between nitrogen fixation and  $pN_2$ ) in presence of  $H_2$  differs greatly from that in the absence of the gas. He further showed that the uptake of combined nitrogen was dependent on the presence of hydrogen in the atmosphere. Wilson and Umbreit [1937] have recently examined this question and have adduced evidence from different experiments to show that hydrogen itself rather than the accompanying impurity is the inhibitory agent. Their findings would suggest that hydrogen may be a specific inhibitor for the symbiotic nitrogen fixation process. This observation is interesting in view of the fact that several workers, notably Stephenson, have

considered hydrogen as a biologically active substance but is quite inert towards non-symbiotic nitrogen fixation reaction brought about by *Azotobacter* [Burk, 1934].

Among the other common enzyme inhibitors concerned with oxidation-reduction reactions in the symbiotic system Wilson [1939, 2] has shown that only CO and H<sub>2</sub>S possess a specific inhibitory effect on symbiotic nitrogen fixation by red clover plants. The effect of CO is more interesting because of the small concentrations required; nitrogen fixation in red clover practically ceases when the concentration of CO in the atmosphere reaches 0.1 per cent.

Among the other enzymes present in the symbiotic system evidence has been obtained to show that the bacterial cells contain gelatinase, catalase, deaminase, carboxylase, tyrosinase, urease, oxidase, peroxidase and various sugar-splitting enzymes [Rajagopalan, 1938]. Eckhardt *et al.* [1931] have observed that *Rh. lupini* produces slight amount of tyrosinase. Almon and Fred [1933] have shown that root nodule bacteria of some cross inoculation groups, notably the bean, alfalfa and soybean groups, showed a higher per cent of cultures producing tyrosinase than did others. The cells contain very little of proteases, toluenated *Rhizobium* culture produce very little proteolysis in vegetable proteins and none at all in cell proteins.

#### NITROGEN FIXATION BY HIGHER FORMS OF LIFE

In recent years increasing evidence has been obtained that the fixation of atmospheric nitrogen is also manifested in some higher forms of plant life. Certain species of algae, yeast, fungi, germinating seeds of legumes and a few other plant cells have been found to fix nitrogen.

##### *Algae*

The investigations of Kruger and Schneidwind [1900] showed that there is no assimilation of free nitrogen in algae. They suggested that probably algae under natural conditions are favourable to the growth of nitrogen-fixing bacteria. Schramm [1914] also found that none of the seven species he experimented with were able to fix atmospheric nitrogen. Wann [1920] has, however, reported that the seven species of grass green algae which were grown in pure cultures in Kjeldahl flasks in mineral nutrient agar containing known amounts of nitrogen (ammonium nitrate and calcium nitrate but not urea, glycocoll, asparagin and ammonium sulphate) fix in presence of glucose 4-13 mg. of nitrogen in five to seven months. Bristol and Page [1923] have, however, failed to confirm Wann's findings. They conducted experiments with four species of green algae in pure culture and they could not find any fixation of nitrogen. They have criticised Wann's methods of analysis as being unreliable.

Moore and Webster [1920] came to the conclusion that unicellular algae can grow and synthesise protein in the absence of all other sources of nitrogen except the elementary nitrogen of the atmosphere, provided CO<sub>2</sub> is present in the medium. Joshi [1928] has reported that the algae in the soil fix atmospheric nitrogen. Drewes [1928] has observed that *Anabaena variables* and *Anabaena* spp. fix 2-3 mg. of nitrogen in 250 c.c. medium in two months. Allison and Morris [1930] have observed that while the species of green algae tested did not fix nitrogen, the blue green algae isolated from soil in pure culture fixed atmospheric nitrogen in presence of light. They [1932] have also shown that the blue green algae *Anabaena variables* fixed appreciable amounts

of atmospheric nitrogen in presence of light and 1 per cent  $\text{CO}_2$ ; soluble nitrogen compounds are produced in the medium. Allison and Hoover [1935, 2] have found that pure cultures of *Nostoc* isolated from soil fixed nitrogen. The dried organism contained 4.6 per cent of nitrogen. Cultures fix 10—20 mg. of nitrogen per 100 c.c. in 50—60 days. In presence of light there was increased growth and nitrogen fixation by the organisms, while in total darkness, nitrogen fixation and chlorophyll formation took place when glucose was supplied to them. They have further shown that the rate of growth and fixation in *Nostoc mucosarium* is 10—20 times greater than that reported for other nitrogen-fixing blue green algae. The quantities of nitrogen fixed are as high as 10 mg. in 45 days and 18 mg. in 85 days per c.c. of a medium containing no carbohydrates. Calcium and strontium are not essential for growth in presence of combined nitrogen, but in nitrogen-free medium, nitrogen fixation is retarded by their absence. Boron and manganese have no effect on nitrogen fixation [Allison and Hoover, 1937]. De [1936] first suggested that fixation of nitrogen in waterlogged soils is an algal process.

More recently, Fritsch and De [1938] have reported that pure cultures of blue green algae *Anabaena* found in Indian rice fields have the property of fixing nitrogen from the air; three species of the organism cultured in nitrogen-free solutions were able to fix 2—5 mg. of nitrogen per 1,000 c.c. medium in about two months. They have claimed that this is the first conclusive proof of the ability of a blue green algae to do so. By repeated sub-culturing on sterilized silica gel plates, De [1939] has isolated pure (bacteria-free) cultures of three species of *Anabaena* and *Phormidium foecolatum*. The *Anabaena* cultures have been found to fix considerable amounts of nitrogen in nitrogen-free medium and soil; small amounts of soil extract in the medium stimulated nitrogen fixation. *Phormidium foecolatum*, on the other hand, afforded no evidence of nitrogen fixation. He has also found that a considerable part of the nitrogen fixed remains in the external medium in an organic form. The author has concluded that algae are the chief agents of nitrogen fixation in the rice fields.

#### *Yeasts, fungi and actinomyces*

Lipman [1910] has shown that certain species of yeasts and pseudo-yeasts have the power of nitrogen fixation in tap water solutions containing dextrose. Kossowicz [1913, 1914] has reported that 5—7 mg. of nitrogen was fixed by *Saccharomyces monilia*, *candida* and *Oidium lactis* in 500 c.c. of non-nitrogenous medium. Fulmer [1923] has shown that *Saccharomyces cerevisiae* will grow in an apparently good state of nutrition using atmospheric nitrogen as the sole source of nitrogen and has suggested that the benefit accruing from the aeration of yeast is as much due to the addition of nitrogen as of oxygen. He has found that fixation of nitrogen by yeasts at  $30^\circ$  is a function of pH, there being two optimal concentrations, the one at pH 6.0 and the other at 7.9, the latter being more potent. The failure to observe any fixation in yeast by different workers has been explained by Fulmer and Christensen [1925] as being due to the time element, and the presence of ring nitrogen compounds which are converted in the early stages into forms not determined by the Kjeldahl method. Christensen [1928] has shown that there is probably more fixation of nitrogen in pure cultures of *Saccharomyces cerevisiae* than what any available method of determination indicates.

Two types of fungi are generally recognized to be of significance in the fixation of atmospheric nitrogen, the free-living fungi and the symbiotic group, known as *mycorrhiza*, which live in the roots of certain higher plants.

Froenlich [1907] found that several strains of fungi fixed from 1.1—4.5 mg. of nitrogen, the amount of nitrogen fixed per gram of dextrose being 2.5—8.9. Stabel [1911] has reported that 9 of the 54 strains studied showed fixation of nitrogen. Schober [1930] has found that all the six strains of *Aspergillus* fixed up to 4 mg. of nitrogen in 100 c.c. medium containing 5 per cent sugar. Kadelbach [1931] and Schroder [1931] have, however, failed to confirm this observation. Chambers [1916] has adduced evidence to show that no nitrogen is fixed by the free-living fungi, *Aspergillus niger* and *Penicillium glaucum*. Waterman [1913], Goddard [1913] and Duggar and Davis [1916] have also obtained negative evidence in regard to nitrogen fixation by free-living fungi. More recently, Allison *et al.* [1934] have concluded that free-living fungi and actinomyces do not fix nitrogen.

There is strong evidence that certain micorhizal fungi can use atmospheric nitrogen when growing in the roots of plants. Rayner [1922] showed that certain strains of *Phoma*, isolated from the roots of ericaceous plants, utilize atmospheric nitrogen. He has also shown that seedlings of *Calluna vulgaris* in pure culture thrive in rooting media deprived of nitrogen. Jones and Smith [1928] obtained similar results and further demonstrated that when the pure micorhizal fungus, *C. vulgaris* grown in presence of molecular nitrogen, uses large amounts of glucose with increase of nitrogen in the medium. Eskina [1938] has suggested that lichens represent symbiosis of three organisms, nitrogen fixing bacteria in addition to fungus and algae, and that the bacteria present are the intragonidial wart-like swellings found in them. In view of the conflicting evidence, it is still doubtful whether the yeasts, fungi and actinomyces are of any importance in nitrogen fixation.

### *Germinating seeds*

Vita [1932, 1] has obtained evidence to show that germinating seeds of legumes have the power of assimilating atmospheric nitrogen. When seeds of pea, lupine or horse bean are germinated in an atmosphere containing CO<sub>2</sub> in presence of alkaloids or even dilute solutions of salts, they absorb nitrogen for their development [Vita, 1932, 2]. Vita and Sandrinelli [1932, 1933, 1935] have studied the various factors that influence this fixation of nitrogen. They have shown that the amount of elementary nitrogen utilized is dependent on the nature and amount of salts, CO<sub>2</sub>, O<sub>2</sub> concentration, temperature and conditions of illumination. They have also observed that in pea and lupine seeds, there is a general relation between nitrogen-fixing and oxidizing power of the seeds. Sugars and some alkaloids like strychnine nitrate and caffeine have marked negative effect. Vita [1935] has found that later in the period of germination the gain in nitrogen partially disappeared. Using lupine and pea seeds, Haritantis [1934] has confirmed these findings. Vita has also postulated that during germination the seeds elaborate an enzyme, azoligase, which is capable of fixing atmospheric nitrogen. The added compounds stimulated its production which resulted in fixation of free nitrogen. The subsequent drop in the nitrogen content of the seedlings has been explained as being due to the action of another enzyme which liberated the nitrogen. Sadasivan

and Sreenivasan [1937] have also observed that there is a progressive increase in nitrogen assimilation in germinating seeds and have suggested that the seeds, independent of any organism, fix atmospheric nitrogen.

Recently, Wilson [1939, 2] has critically examined the available data in regard to the nitrogen fixation by germinating seeds and has questioned the validity of the conclusions arrived at by these investigators. He has shown that the apparent increase in nitrogen which Vita has obtained is due to the inadequacy of the special analytical method used in her studies. The official Kjeldahl method (without addition of water) does not yield all the nitrogen in the seeds. Smith and Wilson [1935] have shown that when a suitable method of analysis was used peas which were germinated under identical conditions prescribed by Vita did not show any gain in nitrogen on germination. Their conclusions are supported by data obtained by the gasometric method wherein also the nitrogen fixation was not apparent beyond the experimental error.

In view of the conflicting evidence it is difficult to draw any conclusion regarding nitrogen fixation by leguminous seeds on germination. Any positive evidence in this direction would no doubt be of much significance in studying the mechanism of symbiotic nitrogen fixation by legumes.

#### *Fixation in other plant cells*

Several workers have reported from time to time that different parts of higher plants exhibit the power of fixing atmospheric nitrogen, either by themselves or by their association with the bacteria present in them. But evidence so far obtained is still inadequate to draw any definite conclusion regarding the relative importance of these as nitrogen fixers.

Lipman and Taylor [1924] have shown that wheat and barley in culture solutions with and without nitric nitrogen fix atmospheric nitrogen without bacterial intervention. Whitley [1923] has also observed that higher plants have got the capacity of fixing atmospheric nitrogen. Moore obtained similar results with other plants. Burk [1927] has shown that the dwarf variety of *Pisum sativum* lost enough nitrogen through excretion in culture solutions to hide any fixation. Brown [1933] has reported that perennial rye-grass meets some of its nitrogen requirements from the atmosphere, especially when nitrogen in the combined form is absent from the medium.

Symbiosis between bacteria and leaves of certain plants was observed in the case of *Pavetta* [Faber, 1912, 1914], *Andrisia crispa* [Miehe, 1914, 1911, 1916] and *Kraussia* [Georgievitch, 1916]. Knots are formed at the place of penetration of the microbes into the tissues of the plant. It is claimed that the bacteria bringing about this transformation can also fix nitrogen when not working symbiotically with the plants. The amount of nitrogen thus fixed may be so considerable that in India *Pavetta* plants are used as green manure. Rao [1933] has shown that the leaf nodules of *Chomelia asiatica* contain colonies of aerobic nitrogen-fixing bacteria. The symbiosis is developed to a greater extent than leguminosae and is of a hereditary character, the plant being unable to grow in the absence of the bacteria. Cauda [1919] has suggested that *Bacillus cruciferae* isolated from various cruciferous plants is found to fix nitrogen, especially when cultured in liquid medium with an excess of calcium carbonate and deficient in nitrogen. The amount of nitrogen fixed is equal to that obtained by *Azotobacter*.

Henry [1904] has reported that dead leaves of various trees fix considerable amounts of atmospheric nitrogen. From experiments with plants grown in nitrogen-free atmosphere Kovessi [1912] has concluded that the plant hairs of phanerogams cannot fix atmospheric nitrogen. Sahasrabuddho [1935] has reported that nitrogen fixation in rice soils is increased by the presence of growing roots of plants. He [1936] has adduced further evidence to show that nitrogen-fixing organisms are active in the presence of rice roots which are good hosts for them. Several investigators [Joshi, 1928; Truffaut and Bezssonov, 1925, 2; Caron, 1923] have also reported fixation of nitrogen by higher plants (barley and corn roots) in presence of various species of bacteria, but no nodule is produced. Oes [1913] has reported that the floating fern, *Azolla*, can grow in nitrogen-free medium and fix atmospheric nitrogen. He further showed that the blue green algae in symbiosis with it accounted for the nitrogen fixation.

#### AGRICULTURAL IMPORTANCE OF NITROGEN-FIXING ORGANISMS

The practical importance of biological nitrogen fixation in agriculture cannot be overestimated. The nitrogen-fixing organisms constitute perhaps the most important factor in maintaining the store of nitrogen in the soil. By proper control of the activities of these organisms it is possible to use these natural agencies—plant and soil bacteria—to supply the nitrogen requirements in the soil for plant growth and crop production.

##### *Role of the different forms in nature*

(i) *Azotobacter*.—The occurrence of *Azotobacter* in the soil is conditioned by three factors, viz. the soil reaction, the soil complex and the available phosphorus content [Gainey, 1925; Wilson and Wilson, 1933; Martin *et al.*, 1937]. Soils having pH value not lower than 6, and available phosphorus particularly in certain proportion to the carbonate content, generally show a good culture of *Azotobacter*, containing 300 per c.c. [Beijerinck, 1921].

Although *Azotobacter* is more potent in tropical climates, it is present in almost all soils of the world. Its occurrence has been demonstrated in most Java soils, in all soils in India, in half of the Polish soils and in about 33 per cent of the cultivated soils of Japan [Hutchinson, 1915; Yamagata and Itano, 1923]. Greaves [1918] has observed that it is very widely distributed in Utah and Danish soils. Dianowa and Woroschilova [1931] have, however, reported that it is completely absent in Finnish soils, even in those that are well buffered and supplied with  $\text{CaCO}_3$ .

The efficiency to fix nitrogen by *Azotobacter* varies with different strains and is markedly affected by seasonal fluctuations [Walton 1915; Vandecaveye, 1938]. Krzenieniewski [1909] has observed that the other soil bacteria have no influence on its activity. Several workers [Konishi and Tsuge, 1933; Walton, 1915; Vandecaveye and Anderson, 1934; Oes, 1913; Bortels, 1937; Ehrenberg, 1910], have observed that the applications of suitable chemicals—zinc and compounds of molybdenum and vanadium and fertilizers, such as basic slag, lime, phosphorus and carbohydrates, increase the number of *Azotobacter* and enhance their activity in the soil. The action of basic slag is not only due to neutralization but also due to the presence of iron and

manganese in them which act as stimulatory agents [Mockeridge, 1914]. Generally speaking nitrogenous compounds depress nitrogen fixation in the soil [Hills, 1917]. Schneider [1931] has, however, observed that the application of  $\text{NaNO}_3$  and urea favours the growth of *Azotobacter chroococcum*, while a continuous dressing of ammonium sulphate retards its development. Baumgentel and Simon [1929] have suggested that the unfavourable effect of much calcium and water in the soil on *Azotobacter* is presumably due to the physiological action of  $\text{Ca}(\text{HCO}_3)_2$  and not due to flocculation of soil colloids.

Our knowledge of *Clostridium* in relation to soil fertility is meagre. Probably these organisms fix nitrogen in the deeper layers of the soil under anaerobic conditions and give off their nitrogen to the plants.

It is fairly certain that non-symbiotic or free-living bacteria considered as a unit, play a definite part in maintaining the supply of nitrogen in the soil by fixing nitrogen from the air. Carter and Greaves [1928], as a result of vegetation experiments lasting over several years, have recorded annual gains of 35 lb. of nitrogen per acre-foot. The estimates made by Hall [1912] as also the later data from Rothamstead would point out to the same conclusion. Several Russian investigators, prominent among these being Kostichev *et al.* [1926], have also stressed the importance of non-symbiotic fixation in the soil. Wilson and Ali [1922] have reported 100 per cent increase in total nitrogen contents of soils in the district of Punjab (India) due to bacterial fixation.

The relative importance of the various non-symbiotic bacteria responsible for the increase of soil nitrogen is not, however, well understood. Most of the European investigators have attributed more importance to the aerobic organisms, especially the *Azotobacter* group. On the other hand, many of the American investigators consider that *Azotobacter* is not so important as the other non-symbiotic organisms. Bonazzi [1915] has shown that there is not yet conclusive proof that *Azotobacter* is of any value under field conditions as a nitrogen gatherer. Waksman [1931] has also reached the same conclusion.

(ii) *Legume bacteria*.—Riede and Bucherer [1939] have found that the soybean nodule bacteria markedly enhance the vegetative and reproductive growth of the plants in nitrogen-poor soil.

Application of nitrogenous fertilizers has a definite influence on nitrogen fixation by nodule bacteria. It has been found that large amounts of nitrate and ammonium sulphate, whether added or accumulated, are injurious to nodule formation in alfalfa, vetch and clover and this in turn on nitrogen fixation [Fred and Gaul, 1916]. Albrecht [1920] has reported that nitrogen fixation by *Pseudomonas radicicola* will take place in soil containing 1,500 lb of nitrogen as  $\text{NaNO}_3$  or 1000-2000 lb. of nitrogen as clover tops per acre. The presence of total nitrogen in the soil up to 3000 lb. per acre does not affect nitrogen fixation by cowpea. Five plants of cowpea in a pot have been found to fix 1295 mg. of nitrogen.

By application of nitrogenous fertilizers, the normally formed legume nodules are rendered entirely inactive [Boidermans, 1918]. The amount of nitrogen fixed is inversely proportional to the amounts of soil nitrogen available to the plant; the effect of nitrate is similar. In early stages nitrogen fixation takes place best when small amounts of nitrogen are supplied to the plant till the flowering stage [Giobel, 1926]. Application of mineral nitrogen

(3 mg. per plant) to inoculated peas kept at low temperatures had very little effect on the amount of dry matter produced. Nitrogenous fertilizers lowered the amounts and percentage of total nitrogen present as protein and amino nitrogen in crops, but the nitrate application somewhat lowered the nitrate content of the crop [Vaitiovaara, 1937]. Thornton [1936, 2] and Thornton and Nicol [1934] have shown that the application of mineral nitrogen does not damage a leguminous crop when grown by itself; the legumes obtain their nitrogen from these compounds instead of through activity of the nodules. However, they adversely affect the growth when the fertilizers are applied to mixed crop of legumes and non-legumes.

Walker and Brown [1935] found that, in general, the application of manure, limestone and phosphate fertilizers to soils served to increase to a large extent the numbers of both the alfalfa and red clover root-nodule bacteria.

It is generally recognized that the leguminous plants are of great economic importance in agriculture. The benefit which they confer on the soil is principally due to the nitrogen compounds elaborated in the root nodules and subsequently released in the soil [Stallings, 1926]. The amounts of nitrogen fixed by various leguminous crops under field conditions have been estimated by several workers and have been found to average to about 100 lb. per acre annually. Analysis of soil under clover, carried by Shutt [1931] over a period of ten years, showed that this crop enriched the soil in nitrogen at an average rate of 50 lb. per acre annually. The amount of nitrogen added to the soil depends on the nature of the soil and the amount of nitrogen available in the soil. The poorer the soil the larger the amount of nitrogen taken from the air. Whiting [1915] has suggested that about two-thirds of the nitrogen in legumes grown on soils of normal productive power is obtained from the atmosphere. On this basis he has estimated that a 3-ton crop of cowpea hay takes 86 lb. of nitrogen, a 25-bushel crop of soybean 106 lb., a 4-ton clover crop 106 lb., and a 4-ton alfalfa 132 lb. The soil enrichment thus produced by legumes may last for several years [Nicol, 1933]. Harrison [1915] has described the preparation of nitro-cultures and their commercial application.

(iii) *Higher forms of life*.—Among the higher forms of life that have the power of fixing atmospheric nitrogen the algae seem to be of some importance. The more recent researches into the fixation of nitrogen by algae have shown that these play an important part in the fixation of nitrogen in the water-logged soils, especially in the rice fields of India.

### *Inoculation experiments*

A good deal of work has been done for increasing the soil nitrogen content by inoculating the soil with suitable organisms. The various attempts made during recent years in this direction may be briefly summarized as follows.

(i) *Azotobacter*.—Emerson [1918] has shown that although inoculation of soil with *Azotobacter* is possible and practicable, it has no effect on the amounts of non-protein, amino or polypeptide nitrogen in the soil and there was no accumulation of these forms of nitrogen in soil under field conditions. Lipman [1908] and Lipman and Brown [1907] have reported that inoculation with *Azotobacter* in presence or absence of organic matter decreased rather than increased the yield, dry matter and nitrogen content of corn crops. Bottomley [1910] has, however, obtained increased yield in pot experiments

with barley, *Avana sativa*, galtonia and parsnips by inoculating seeds with mixed culture of *Azotobacter chroococcum* and *Pseudomonas radicicola*. He [1914] has also shown that when specially treated peat containing nitrogen-fixing organisms are added to soils in pots, there is increase in total nitrogen of the soil resulting in large increased growth of a variety of plants. Stoklasa [1909] has obtained better yields of oats, potatoes and beets by soil inoculation in field experiments. Brown and Hart [1925] have found that wheat yield was not increased although nitrogen accumulated in the soil as a result of inoculation with *Azotobacter*. Inoculation with 'nitrofer' has been found to be effective in increasing nitrogen fixation in the soil [Zucker, 1928]. Makrinoff [1929] has reported good results from inoculation with non-symbiotic bacteria. More recently, Karunakar and Rajagopal [1937] have obtained significant increase in yield of grain and straw in sorghum by inoculation of seeds with *Azotobacter*; there was greater response by the addition of  $\text{CaCO}_3$  and  $\text{K}_2\text{HPO}_4$ . Martin and Brown [1937] have, however, found that inoculating with *Azotobacter* increased the dry weight and the total nitrogen in timothy grass but not in corn and wheat.

For successful inoculation suitable carbohydrate, sufficient air supply, lime,  $\text{P}_2\text{O}_5$ ,  $\text{K}_2\text{O}$  and other essential mineral nutrients in the soil are necessary [Stoklasa, 1909; Vandecaveye and Anderson, 1934; Omeliansky, 1915]. Kreybig [1929] has emphasized the importance of soil reaction for successful inoculation. There was greater bacterial activity in inoculated lime plots [Martin and Brown, 1937]. Eugel [1931] has shown that *Azotobacter* nitrogen (dead or alive) is easily nitrified in the soil.

(ii) *Legume bacteria*.—Ball [1907] has found from pot experiments that in artificial inoculation of 'nitroculture' the number and vigour of the tubercles were not as great as that occurring by natural means. Inoculation of seed with nitroculture leads to increase in nitrogen; potassium and phosphorus fertilizers gave a further increase [Wright, 1908]. Soil and seed inoculation with nitrugin and nitrobacterine increased yield in lupines and sand peas, especially in the presence of phosphate. The nitrobacterine showed a greater effect than nitrugin in soil poor in lime [Grabner, 1910]. Inoculation of undecomposed virgin Shapgnum moor soil with nitrugin and azotogin are found to have very good action on yellow lupines; farmogen was almost inactive [Ferlitzén and Nystrom, 1914]. The soil conditions for the application of nitrugin are deficiency of nitrogen, soil reaction and presence of sufficient amounts of other fertilizing ingredients and pH [Anon, 1919]. Growth of lucerne in south-east Scotland soil took place between pH 6 and 7; and no growth was observed between pH 5.0 and 5.49. Inoculation with bacteria resulted in an increase of 100 per cent total nitrogen of dry matter. Some strains were more effective than others [Cunningham, 1928]. Nolte [1919] has reported that three of the bacterial nitrogen fertilizers that he tried are found to be of limited value as sources of supplying nitrogen to the soil.

Brown and Stahlings [1921] have found from pot experiments using inoculated clover and alfalfa that when the hay crops are removed, there may be some gain in nitrogen in the soil. Inoculation of the nodule bacteria of *Cicer* increased the nitrogen content and produced increased crop yield [Razwumovskya, 1934]. Practical legume inoculants containing two or more

legume cultures compare favourably with standard group single cultures with respect to efficiency of nitrogen fixation and nodule formation [Bond, 1938].

Light, heat and exposure do not affect soil cultures as much as agar or liquid cultures [Fellers, 1919]. Drought and heat have only a temporary effect on legume bacteria in Poulouse silt loam [Vandecaveye, 1925]. The poor development of nodules in acid soil is due to the effect of acidity on the bacteria during the interval they exist non-symbiotically [Karrakar, 1927]. Vandecaveye [1927] has shown that *Rh. leguminosarum* is capable of surviving wide extremes of moisture and long periods of absence of host plant; it is distributed by wind and dust storm to a slight extent. Soybean nodule bacteria is relatively short lived, rarely surviving a year [Wilson, 1934]. Movement of *Astragalus sinicus* (genge) is largely influenced by moisture content (optimum being 18 per cent and no movement at less than 5 per cent) and silt concentration. There is strong chemotoxic action between bacteria and genge seeds [Itano and Matsuura; 1934]. Hofer [1938] has shown that five to forty bacterial cells are necessary for successful inoculation of clover and alfalfa seeds. Asparaginate in place of yeast extract is useful in the medium for distribution of cultures. For best performance, the culture for inoculation should carry at least 80 millions of bacteria per lb. of seeds.

Joshi [1920] has found that the root nodule bacteria exert a beneficial influence on graminaceous plants also. By experiments with porous cylinder he has shown that soluble products are excreted into the soil. Stahlings [1926] has shown that wheat grown with inoculated soybeans may under favourable conditions obtain considerable amounts of nitrogen from the latter, with a lowering in their nitrogen content. It contained a higher percentage of nitrogen than wheat grown alone. Decrease in acidity of soils leads to increase in nitrogen content in leguminous plants; at pH 6 it is 30 per cent higher than at pH 5. Demelon and Dumez [1938] have shown that the same soil fatigue phenomna due to continued legume culture occurring with clover, lupine, peas, beans and soybeans is the same.

A thick close crop in crimson clover favours an early accumulation of nitrogen, the first month of growth yielding one third the total. The distribution of nitrogen in different parts of the plant varies greatly, about a third on an average being in the root [Penny and Macdonald, 1909]. Smith [1912] has reported that the number of *Rhizobia* present varies from 3—4 millions per gram of soil; the number of the organisms affords an index of the fertility of the soil. Application of nitrogenous manure during the early period of growth of inoculated plants produce good results by preventing any injury during the period of hunger [Ritter, 1911].

(iii) *Seed, root and plant inoculation.*—Dunham and Baldwin [1931] have indicated the necessity of using only effective strains of the nodule organism, for seed inoculation since definite detrimental results may occur by the use of ineffective strains. Nobbe *et al.* [1909] have found that pure culture of bacteria obtained from a kind of legume works symbiotically with other species of the same genus of plants. There are, however, genera of legumes, such as, pea and vetch, serradella and lupine, in which reciprocal inoculation increases the supply of nitrogen in the soil.

Inoculated seeds should not be stored for long periods before sowing, but the delay of several days or even a month may not do great harm [Fellers, 1918].

From a comparative study of the nitrogen content of seeds and inoculated plants Whiting and Schooner [1920] have shown that marked fixation of nitrogen takes place after the formation of the first leaf, 19 days after planting. The first appearance of nitrogen fixation was nine days after planting and by 26 days the amount of nitrogen fixed was three times that contained in the original seed. The older seeds of a given legume are more acid than the fresh seeds [Wilson, 1939, 1]. Thornton [1929] has shown that the appearance of nodules coincides with the opening of the first true leaf. The active substance inducing nodulation is not formed by the leaf, for the removal of leaf while still closed has no influence on nodule appearance. Plants containing sufficient nitrogen are immune to further infection of *radicicola* [Lohnis, 1930]. Link [1937] has shown that B-indolacetic acid is one of the agents, if not the agent, responsible for the incitation of nodulation in susceptible hosts. The effect of such hetero-auxones may account for the beneficial effect obtained by green manuring with nodule forming plants and by manures, composts and humus soils. Chemicals have very little effect on nodulation. Treated seeds produce larger nodules than the untreated [Kadow *et al.*, 1937].

#### *Mixed culture studies*

So far, few attempts have been made to study nitrogen fixation by the mixed flora of the soil which is the nearest approach to soil conditions. The precise manner in which the nitrogen-fixing organisms, especially *Azotobacter* and *Clostridium* function in the soil where they have to compete with the other soil organisms and the extent to which the combined activities of all these organisms contribute towards nitrogen fixation in the soil are not well understood. The recent investigations of Bhaskaran and Subrahmanyam [1937] with the mixed flora of the soil have shown that the study of nitrogen-fixing organisms in pure cultures are only of limited value in explaining the mechanism of the process occurring in the soil. They have reported that the fixation of nitrogen by the mixed flora of the soil follows a different course from that of a pure culture of *Azotobacter* alone in artificial media. The latter is comparatively slow in decomposing sugar, and the fixation proceeds only so long as the sugar lasts in the medium. The residual matter is not utilized to an appreciable extent in the fixation of nitrogen. On the other hand the mixed flora of the soil though fewer in number rapidly decompose the sugar. Only a small quantity of nitrogen is fixed in presence of sugar while the major part amounting to over two-thirds of the total quantity fixed, is fixed in the later stages [Bhaskaran and Subrahmanyam, 1936]. They [1937] have further shown that the products of decomposition of sugar are utilized in this subsequent fixation.

The above observations would suggest that although *Azotobacter* may be potent by itself in the early stages of sugar decomposition, it does not play a large part in nitrogen fixation in presence of other organisms of the soil. The latter decompose the sugar at a rapid rate, so that it will receive only a limited amount of the organic nutrient and will, in consequence, fix only a small amount of nitrogen. The fixation that takes place in the soil after the disappearance of sugar is presumably due to the other organisms present in the soil.

Bhaskaran and Subrahmanyam [1937] have also shown that the residue left after the decomposition of sugar is highly potent in fixing nitrogen in the soil. Thus, there is threefold increase in the amount of nitrogen fixed by the mixed flora of the soil when the residue is used as energy material in place of sugar. From the agriculturists' point of view, this observation is of considerable practical importance.

### DISCUSSION

From the evidence so far collected on the question of fixation of atmospheric nitrogen by living forms, it would appear that the capacity of fixing nitrogen is mainly confined to unicellular organisms. The distribution of nitrogen-fixing power is not, however, manifested in any one stage of evolution of life. Perhaps the algae are the most primitive organisms which have the power of fixing nitrogen. Probably the processes of carbon and nitrogen assimilation, as manifested in the blue green algae *Anabena*, are coeval in the process of evolution and this must have been the case in order that any living organism could have ever appeared on this earth. The fact that organisms living under entirely different environmental conditions, such as, in the absence of molecular oxygen, in presence of plenty of oxygen and in the living tissues of higher plants fix nitrogen, would show that the function of nitrogen fixation was more universal among the living organisms once upon a time. Later on, with evolution only a few of them retained the power so as to maintain the stock of combined nitrogen in the soil.

The process of fixing elementary nitrogen is not, however, identified with the life of the organism. The organism does not fix nitrogen in presence of readily available combined nitrogen in the medium. Further, they do not depend on the nitrogen-fixing process (unlike the autotrophic bacteria which depend on nitrification) for their maintenance. The process of nitrogen fixation being a secondary life process, it is probable that the fundamental mechanism involved in nitrogen fixation is similar in the different forms. The study of the chemical changes that are involved in nitrogen fixation apart from the general metabolism of the organisms has been of great scientific interest.

The nitrogen-fixing reaction requires a source of energy, the presence of minerals Ca (replaceable by Sr) and Mo (replaceable by V) and an optimum reaction. In view of the uncertainty of the first chemical step in nitrogen fixation it is not known whether the process is exothermic or endothermic. Whether it fixes nitrogen or not the organism uses the same amount of energy material (carbohydrate) for its growth. These observations would suggest that the energy material has no part to play in the chemical mechanism of nitrogen fixation. It is, however, difficult to understand how small concentrations of Ca and Mo (replaceable by Sr and V respectively) have a specific role in nitrogen fixation.

The nitrogen-fixing reaction in the living forms is essentially a chemical process. The changes of the  $N_2$  molecule to form part of the bacterial protein are so rapid that it has not been possible to know the different stages of the reaction. To get a complete picture of the scheme of reaction, it is necessary to isolate the nitrogen-fixing apparatus apart from the living cell so that the reaction could be arrested at the different stages and studied.

A consideration of the physico chemical data in regard to nitrogen fixation would show that the catalyst responsible in bringing about the reaction is an enzyme. The extra-cellular isolation of the enzyme has not, however, been possible. The future line of work therefore consists in the isolation of this enzyme and study of its exact chemical nature with a view to finding out the active chemical group in it which is ultimately responsible for the nitrogen-fixation reaction.

In addition to its theoretical interest the problem of biological nitrogen fixation is of considerable practical importance. So far, the various attempts to use the nitrogen-fixing organisms as a source of supplying nitrogen to the soil for increased plant growth has not been successful. Probably a fuller understanding of the chemical mechanism of nitrogen fixation would enable the farmer to make use of this biotic energy for increased crop production.

### SUMMARY

1. The various forms of life that fix atmospheric nitrogen in nature have been classified into (a) the non-symbiotic or free living bacteria, (b) the symbiotic or legume bacteria and (c) higher forms of life consisting of algae, fungi, yeast, actinomycetes, germinating seeds of legumes and different vegetative parts of certain higher plants.

2. *Azotobacter* and *Clostridia* are the important non-symbiotic organisms which fix nitrogen in the soil. The *Azotobacter* is typical of the aerobes and the *Clostridia* of anaerobes.

3. *Azotobacter* is generally present in soils having pH above 6.0. The cells contain volutin bodies, fat and metachromatic granules.

4. The nutritional requirements of *Azotobacter* consist of a source of energy, water and certain minerals. The organism uses carbohydrates, salts of organic acids and alcohols as energy source. Soil humus has been found to exert a stimulatory influence on the organism for nitrogen fixation. Vitamin B<sub>1</sub> and phytonucleic acid stimulate growth and nitrogen fixation. Certain minerals in optimum concentration are necessary for the growth of *Azotobacter*, and among these calcium (replaceable by strontium) and molybdenum (replaceable by vanadium) are specific for nitrogen fixation; manganese and uranium compounds accelerate nitrogen fixation. Iron plays no specific role in the mechanism of nitrogen fixation.

5. *Azotobacter* respire at an enormously high rate. Spectroscopic examination of the cells reveals a respiratory mechanism similar to those ascribed to aerobic cells: the respiratory enzyme band is in the red region at 632  $\mu$ .

6. The activity of *Azotobacter* is dependent on the reaction of the media, temperature and air supply. The organism grows between pH 6.0 and 9.6. It grows and fixes nitrogen between temperatures 10 and 50°C., the optimum being between 34 and 35°C. Aeration of the medium facilitates nitrogen fixation.

There is, however, a marked influence on the amount of nitrogen fixed when the organism is exposed to light of different colours; yellow light is better than blue. It fixes more nitrogen in presence of protozoa, amoeba and certain species of bacteria and algae.

7. The organism does not fix nitrogen in presence of rapidly available combined nitrogen, 0.5 mg. per 100 c. c. media completely inhibits nitrogen fixation.

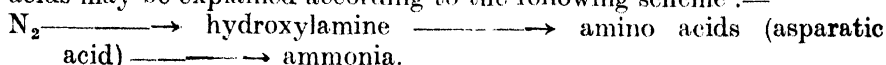
8. In dextrose media, *Azotobacter* produces formic, acetic, lactic and tartaric acids and ethyl alcohol; a large part of the sugar is converted into carbon dioxide.

9. The cells consist chiefly of carbohydrates, proteins and a small percentage of minerals. The proteins are very similar to those of other organisms except for a high content of arginine.

10. In culture media *Azotobacter* produces a characteristic slime. It is a carbohydrate, and is levo-rotatory and it belongs to the class of true gums.

11. With ageing in culture medium the organism produces characteristic pigments; the pigment is a melanin of unknown nature formed from tyrosine by the action of tyrosinase.

12. Nitrate, ammonia, hydroxylamine and amino acids have been reported by different workers as the first intermediate product in the fixation of nitrogen by *Azotobacter*. Direct experimental evidence for the oxidation of nitrogen to nitrate is lacking. Detection of ammonia, hydroxylamine and amino acids may be explained according to the following scheme:—



It is therefore likely that the first intermediate product is hydroxylamine and that the amino acids and ammonia detected represent the later stages of the reaction.

13. The properties and behaviour of the nitrogen-fixing system in *Azotobacter* are, however, characteristic of an enzyme reaction. It has been considered as a phycoenzyme and named azotase. The specific component within the azotase system which combines with the  $\text{N}_2$  molecule is termed nitrogenase. The auxillary substances known at present for the enzyme reaction are calcium (replaceable by strontium) and molybdenum (replaceable by vanadium) and hydroxyl ion. The extra-cellular isolation of azotase and the study of the enzyme apart from the growth and general metabolism of the organism has not so far been possible.

14. *Clostridia* are present in almost all soils of the world and are found in rather large numbers in acid soils. They occur more abundantly than *Azotobacter*.

15. The different species of *Clostridia* have not been clearly defined. *Clostridium pasteurianum* is typical of the species fixing nitrogen, and it is an obligate anaerobe.

16. The organism can be easily isolated from soil by using Winogradsky's nutrient medium and by prolonged pasteurization at 75°C. The optimum temperature for the development of *Clostridium pasteurianum* is between 28° and 30°C. and the optimum reaction is between pH 6.9 and 7.3.

In presence of oxygen the organism forms characteristic spores. The spores are not destroyed at 75°C. even at the end of 15 hours. They could be preserved in the dry state for 20 years with the nitrogen-fixing power intact.

17. In sugar media characteristic butyric fermentation takes place; 42—45 per cent of dextrose is converted into a mixture of acetic and butyric acids in varying proportions; small amounts of alcohol (ethyl, propyl and

isobutyl) are formed and a considerable evolution of a mixture of carbon dioxide and hydrogen also takes place.

18. In nitrogen-free media the organism fixes about 3 mg. nitrogen per gm. of sugar decomposed. The greater the concentration of sugar, the lower is its economic utilization ; 3.2 mg. nitrogen is fixed in 0.5 per cent glucose solution, 2.0 mg. in 2 per cent solution and 1.2 mg. in 4 per cent solution.

Combined nitrogen in the medium reduces nitrogen fixation ; presence of 6 parts of combined nitrogen in 1,000 parts of medium has been found to completely inhibit nitrogen fixation.

19. It is claimed that a large number of free-living bacteria present in the soil other than *Clostridium* and *Azotobacter* have the power of fixing nitrogen. But it is doubtful whether these are of any importance in soil nitrogen fixation.

20. A group of soil bacteria known as *Rhizobia* has been found to infect the roots of leguminous plants and develop characteristic nodules ; the bacteria in association with the plant fix atmospheric nitrogen.

Six species have been recognized in this group of bacteria, viz. *Rh. leguminosarum* Frank, *Rh. trifolii*, *Rh. phaseoli*, *Rh. melilote*, *Rh. japonicum* and *Rh. lupini*.

21. Whether grown in culture media or soil, the bacteria exhibit a clear and definite life cycle which consists of five stages : (a) the small non-motile pre-swarmers coccus, (b) the larger non-motile coccus, (c) motile swarmer, (d) rod form and (e) the stage of high vacuolation (bacteriod).

This distinct life-cycle has a bearing on the spread of bacteria through the soil and consequently on the infection of the host plant. Special reproductive cells, gonidia or spores are not formed in the process of reproduction.

22. The nodule bacteria can be easily cultivated in artificial media. The optimum pH for the growth of the nodule bacteria in nutrient gelatin lies between 6.5 and 7.5. The bacteria present in different leguminous plants are divisible into two physiological groups according to their cultural characteristics and biochemical reactions. The organisms present in alfalfa, clover, pea and dahlia produce an acid reaction in sugar media while those of soybean, cowpea and lupine an alkaline reaction.

23. The appearance of the nodule on the seedling takes place with the unfolding of the first true leaf ; the removal of the leaf, however, does not delay nodule formation. The active substance secreted by the bacteria produces a weakening of the cell-wall and bacteria enter the root at this point.

There is a marked specificity in the infection of host plant by bacteria ; 18 host specific species have so far been recognized. Infection of the host plant outside the specific group is of rare occurrence.

24. The entry of the bacteria into the root hair produces important histological changes in the host tissue leading to rapid division of the root cells and formation of nodular tissue. The bacteria are distributed through the end nodules in three different ways in different legumes : entry through perforation in the cell wall, infection of the inter-cellular spaces and invasion of the meristematic cells. The cytoplasm of the infected cells becomes closely packed with bacteria which later on become branched and constitute the so-called ' bacteriods '. A group of these bacteriods form the nodule.

25. Neither the bacteria nor the host plants can fix nitrogen by themselves. The process of nitrogen fixation is therefore the result of symbiotic relationship between the plant and the bacteria. The nitrogen fixed in the nodular tissue is translocated to the different parts of the plant.

26. The proper functioning of the bacteria within the host depends upon the maintenance of a nice physiological equilibrium between the host and the bacteria. Presence of nitrate, ageing of the nodules and food supply determine this equilibrium. An adequate and unhindered carbohydrate supply is essential for the healthy functioning of the nodule.

27. Natural humic acid and iron salts stimulate growth of *Rhizobia*. Adsorbed calcium is useful in transforming the abnormal nodules into effective nodules. Titanium salts have specific morphological influence on the bacteria. The supply of exchangeable bases is a limiting factor in legume growth and nitrogen fixation.

28. Maximum growth and respiration take place near neutrality with constant activity between pH 6.0 and 7.8. The effect of soil acidity on nodule formation is not due to its effect on the growth of roots of the plant but due to its effect on bacteria when it exists in the soil non-symbiotically.

29. Yeast extract, molasses, sauerkraut and extracts of leguminous plants contain accessory growth substances which greatly influence the growth of nodule bacteria. They provide an initial H donator which in turn lowers pH and supplies a readily available initial source of energy.

These extracts contain a secondary accessory factor which in conjunction with vitamin B<sub>1</sub> is highly active in promoting the growth of bacteria. This effect is due at least in part to the presence of thiamin and flavin in those products.

The beneficial effect of certain extracts may be due to their effect on the oxidation-reduction potential of the medium.

30. The formation of bacterioids in the nodosities is also dependent on the presence of alkaloids in the roots. Caffeine, quinine and strychnine in small doses stimulate growth of bacteria in liquid cultures.

31. The bacteriophage isolated from nodes of leguminous roots is active in dissolving the bacteria; the lytic action is specific.

32. The presence of rapidly available combined nitrogen in the soil decreases nitrogen fixation in the nodules; the influence depends on how it alters the C : N relationship in plants.

Small amounts of amino acids result in the loss of infective ability of *Rhizobia*.

33. With nitrogen fixation the organism produces a characteristic slime. It is produced in faintly alkaline and neutral media but not in acid media. It is a polysaccharide containing glucuronic acid.

34. The composition of the nodular tissue is not different from that of the other parts of the plant. The proteins are similar to those of *Azotobacter*. 20 per cent of the nitrogen of the nodule is arginine.

35. In sugar media the bacteria produce acids, alcohol and traces of aldehyde; the fermentation is of the pyruvic acid type.

36. Evidence has been adduced to show that in symbiotic nitrogen fixation nitrogen molecule is first converted into hydroxylamine through an unknown intermediate. The hydroxylamine reacts with the oxalacetic acid

from the plant to form oxime which is then reduced to give *l*-aspartic acid. But the evidence in support of this scheme of reaction is, however, inadequate.

37. The nitrogen compounds excreted by nodules are mainly amino acids; aspartic acid accounts for 50 per cent of the amino acids, while the other half probably is lysine. The excretion takes place right from the bacteria inside the nodules and not from the roots. Direct contact of the roots and nodules with solid particles is necessary for nitrogen excretion. The excreted nitrogen represents the primary product of nitrogen fixation and not the decomposition product of the proteins.

38. The rate of respiration of nodule bacteria is considerably higher than that of other bacteria. The respiratory quotient (*R. Q.*) is higher in *Rh. trifolii* than in the other species. The rate of oxygen consumption increases with increased oxygen pressure in the atmosphere.

Yeast extract, cane molasses, humic acid and commercial egg albumin increase the rate and extent of oxygen consumption by all strains of *Rhizobia*. This may be due to the presence of a coenzyme in those substances, which is essential for respiration.

39. Symbiotic nitrogen fixation has also been considered as an enzyme reaction. Nitrogen fixation is dependent on the pressure of nitrogen ( $pN_2$ ); the thermodynamic dissociation constant ( $kN_2$ ) is  $0.05 \pm 0.005$  atmospheres, whereas in the non-symbiotic organism (*Azotobacter*), it is  $0.215 \pm 0.002$ . The relation of the nitrogen-fixing reaction to oxygen pressure being independent of the source of nitrogen, it is probable that molecular oxygen is not directly concerned in the fixation process. In presence of  $H_2$ , however, there is an intimate relationship between the enzyme system responsible for fixation of free nitrogen and the oxidative system present in it. Molecular hydrogen inhibits symbiotic nitrogen fixation whereas it is quite inert towards non-symbiotic nitrogen fixation brought about by *Azotobacter*.

40. Certain species of algae, yeast, fungi, germinating seeds of legumes and certain plant cells have been found to fix atmospheric nitrogen.

41. Three species of the blue green algae, *Anabaena* isolated from the rice fields of India have been found to fix atmospheric nitrogen.

42. The evidence for fixation of nitrogen by free living fungi, yeasts and actinomyces is, however, inadequate. But mycorrhizal fungi in association with the roots of ericaceous plants utilize atmospheric nitrogen.

43. In view of the conflicting evidence it is difficult to draw any definite conclusion regarding the ability of germinating seeds of legumes to assimilate atmospheric nitrogen.

44. It is claimed that roots and leaves of certain non-leguminous plants exhibit the power of fixing atmospheric nitrogen either by themselves or by their association with the bacteria present in them.

45. The nitrogen-fixing bacteria in the soil are the chief agents responsible for maintaining the store of soil nitrogen.

46. The relative importance of the various non-symbiotic bacteria responsible for the increase of soil nitrogen is not well understood. *Azotobacter* is present in almost all soils of the world and is more potent in tropical soils. Our knowledge of *Clostridium* in its relation to soil fertility is, however, meagre; probably this fixes nitrogen anaerobically in the deeper layers of soil.

47. The leguminous plants are of great economic importance in agriculture. The nitrogen fixed in their nodules is released in the soil for plant nutrition. The soil enrichment thus produced by legumes may last for several years.

48. Artificial inoculation of *azotobacter* and other non-symbiotic nitrogen-fixing organisms in the soil has not so far proved successful in general agricultural practice.

49. The various attempts at soil, seed and plant inoculation with commercial cultures of legume bacteria are described. It is possible by seed inoculation to improve the growth of legumes in regions where non-effective strains of nodule bacteria predominate in the soil.

50. The fixation of nitrogen by the mixed flora of the soil follows a different course from that of pure cultures of the nitrogen-fixing organisms in artificial media. The economy of carbon utilization in nitrogen fixation by the mixed flora of the soil is different from that of *Azotobacter* in pure culture.

When the products of anaerobic decomposition of sugar are used in place of sugar as energy material, there is threefold increase in the amount of nitrogen fixed by the mixed flora of the soil.

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### ADDENDUM

Since this paper was communicated for publication, a large volume of work has been done, particularly on the biochemical aspects of nitrogen fixation. A brief reference may be made to the more important of these researches.

#### *Azotobacter*

Bortels [1939 ; 1940] and Burk and Horner [1940] have further shown the need of molybdenum in growth by *Azotobacter*. At the same time it is becoming increasingly clear that although all nitrogen-fixing organisms so far tested require molybdenum (or vanadium), iron, and calcium (or strontium), in no case can it now be considered as probable that these elements are specifically required in the nitrogen-fixation process as distinguished from general assimilation of combined nitrogen. The only qualitative fixation specificity that can be regarded as established at present is hydrogen inhibition, and even this is probably essentially physical rather than chemical.

Burk [1941] has shown that growth of *Azotobacter*, in both free and fixed nitrogen, requires, as in the case of most if not all other bacteria and higher forms, a minimum concentration of carbon dioxide ; inhibition of *Azotobacter* growth by too low pressures of carbon dioxide (0.05 per cent or less) is readily observed in a Warburg apparatus by maintaining too effective absorption of respiration carbon dioxide in the alkali, with very dilute cultures. Carbon dioxide about 1 per cent or more lowers (reversibly) the concentration range over which nitrite is toxic for respiration and growth.

Wyss and Wilson [1941] have reported that essentially all the findings on inhibition of symbiotic fixation by hydrogen [Wilson, 1940] hold also for fixation by *Azotobacter*. Burk and Burris [1941] have confirmed these observations, which obviously introduce great unity into our conception of the processes of fixation in *Azotobacter* and in legume symbiosis. Acceptance of these new findings necessitates a re-interpretation of the hyperbolic function obtained in the experiments of Lineweaver, Burk and Deming several years ago.

Horner and Burk [1939] have observed that young cultures of *Azotobacter* vigorously fixing nitrogen generally excrete some 10-25 per cent of the nitrogen into the surrounding medium. The extra-cellular nitrogen is quite a heterogenous mixture: about two-thirds is precipitable by lead acetate, one-third by phospho-tungstic acid, one-fifth by aluminium sulphate and still less by trichloroacetic acid. Winogradsky [1939, 1, 2], in support of his previous observations, has adduced evidence that autolyzing silica gel cultures of *Azotobacter* yielded more ammonia nitrogen after disappearance of the organic substrate than corresponded to the simultaneous loss of total nitrogen. In his opinion, a highly efficient enzymic synthesis of ammonia was involved, which accumulated slowly over a period of months a relatively important amount of ammonia, under conditions that might well obtain in soils or waters. The evidence is still not convincing.

#### *Clostridium*

The present authors have obtained evidence that during decomposition of glucose by *Clostridium pasteurianum*, there is very little fixation of nitrogen in the first 10-12 days, although during this period there is rapid conversion of the sugar carbon into volatile acids and other soluble products. The major part of the fixation takes place only after the sugar has disappeared from the medium. During this period there is little loss of carbon from the medium, the efficiency of nitrogen fixation being of the order of 1:19. There is greater intake of carbon than nitrogen by the cells during the early stages of growth. The nitrogen thus fixed is mostly present in the residue consisting of bacterial cells.

It may, therefore, be concluded that nitrogen fixation by the mixed flora of the soil in sugar media takes place in two stages: the first stage of aerobic fixation which continues till the added sugar disappears and in which *Azotobacter* is the chief organism concerned in the fixation; this is followed by the second stage in which *Clostridium* contributes the nitrogen fixation. From the point of view of carbon utilization, the efficiency of nitrogen fixation in the later stage is much more favourable than in the first stage. It would appear that *Clostridium* is of greater importance than *Azotobacter* in soil nitrogen fixation.

#### *Rhizobium*

In his monograph Wilson [1940] has dealt with the different aspects of symbiotic nitrogen fixation (chiefly biochemical) that have undergone considerable development in the last decade—the biochemistry of the bacteria in pure culture, the interaction of host plant and bacteria, the chemical mechanism of the fixation process, the effect of the carbohydrate-nitrogen balance in the host on fixation, excretion of nitrogenous substances from nodules into the rooting medium, physico-chemical studies of possible enzyme systems, and a discussion of practical applications.

Virtanen [1939] has claimed that legumes utilize aspartic acid in preference to all forms of fixed nitrogen, and that non-legumes (wheat, barley) use it scarcely at all, a claim of great significance for the hydroxylamine (oxime) theory of legume nitrogen nutrition. The weakness of this theory is that neither hydroxylamine nor oxime has ever been clearly shown to be an available source of nitrogen for nutrition of either symbionts or *Azotobacter* even at non-toxic concentrations.

Virtanen and Laine [1939], and Virtanen and Törnainen [1940] have further shown that under the widely varied and studied experimental conditions used by them leguminous plants almost invariably excrete into the soil considerable amounts of the nitrogen fixed from the atmosphere. The amounts excreted are greater than can be accounted for by sloughing-off of nodules, and excretion frequently occurs early in the life of the plant before appreciable decay or sloughing-off would be anticipated. The objection that non-symbiotic nitrogen fixation is responsible for the observed effect is answered by experiments carried out by Virtanen and his co-workers under bacteriologically controlled conditions. Wilson and Wyss [1939], Bond and Boyes [1939], Romashev [1939], and

Ludwig and Allison [1940] have obtained negative results. Slight, but questionable, excretion has been observed by Shapter [1939] and by Madhok [1940]. Variable results with some positive findings have been recorded by Scholz [1939]. Wilson [1940] concludes that excretion is obtained only under particular conditions, namely, those providing sufficient photosynthesis to ensure a fairly high rate of nitrogen fixation but without excess of carbohydrate to bind into the plant all nitrogen as it is fixed.

According to Kubo [1939] the 'red body' present in nodules, which Pietz described as an oxidation product of dihydroxyphenylalanine, is a hemoprotein. He found that the hemoprotein, which he has isolated from the nodules of many leguminous species, on dissociation, gives a hemin identical in crystal form with the hemin from horse hemoglobin. He reported that the hemoprotein stimulated succinate oxidation by *R. japonicum*. Link and Eggers [1940] found that nodules of beans and peas had different auxones and a higher auxone content than roots grown on sterile substrates. Georgi and Beguin [1939], however, found that the soil organism *B. radiobacter* produced auxin at a more rapid rate than the root nodule bacteria. Evidence that auxins are really critical in the formation of the highly differentiated tissue of root nodules is as yet merely suggestive.

In their initial publication Allison, Hoover and Burk a few years ago observed that coenzyme R is not identical with the complex, bios, and left open the question of its relationship to components thereof. Nilsson *et al.* [1939, 1, 2], and West and Wilson [1939; 1940] showed its virtual identity with bios IIB, or biotin. Still further confirmation was later provided by Gyorgy *et al.* [1940, 1], who concluded that these two substances were also identical with vitamin H, a conclusion that was further established by Vigneaud *et al.* [1940] and Gyorgy *et al.* [1940, 2]. The establishment of the identity of biotin, coenzyme R, and vitamin H is obviously of great significance for connecting plant and animal vital economy, the more so in view of the already demonstrated rôle of coenzyme R in respiration and hence probably in fundamental intermediate metabolism, and also in view of the many directions which research on biotin has taken in 1940, since its connection with animal metabolism has become known.

So far, no direct rôle of coenzyme R is indicated in the nitrogen-fixation process. This possibility is not to be excluded, however, in view of the fact that the effect of coenzyme R in *Rhizobium* respiration involves the presence of readily available nitrogen [Allison and Hoover, 1939], as has also been demonstrated by Burk *et al.* [1941] in its effect on both respiration and fermentation of yeast.

Ruben *et al.* [1940] have made an isotopic approach to the study of nitrogen fixation. They exposed tops of barley plants to purified radioactive nitrogen,  $N^{15}$ , for 20 minutes, and then subjected the tops to a hot alcohol extraction; the extract was next boiled in a stream of air. An extract from a control plant, killed by boiling water, showed no radioactivity, whereas the extract from the live plant contained small amounts of  $N^{15}$ . As the authors stated, the evidence indicates fixation of nitrogen, but the possibility of exchange has not been eliminated, and more details and control experiments are needed for the data to be convincing.

### Algae

Referring to the recent work of De [1939] suggesting that certain algae, particularly those from some rice fields in Bengal (India), are able to fix atmospheric nitrogen, Chaudhuri [1940] has reported that his own work leads to the view that bacteria (*Azotobacter*), living in the mucus sheath of these algae, are responsible for nitrogen fixation.

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# STUDIES WITH WHEAT UNIFORMITY TRIAL DATA

## I. SIZE AND SHAPE OF EXPERIMENTAL PLOTS AND THE RELATIVE EFFICIENCY OF DIFFERENT LAY-OUTS

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(With two text-figures)

THE conclusions of any field experiment are based mainly on the pooled estimate of the variance within the different replications after eliminating for treatment effects. This variance, as is well known, is influenced by a number of causes, the most important of them being the size and shape of plots, the design of the lay-out and the extent of heterogeneity present in the site selected for the experiment. Uniformity trials, as indicated by Cochran [1937], enable us to improve the methods of field experimentation by obtaining information on the three points mentioned above.

A review of the literature on uniformity trials will show that most of them deal with the optimum size and shape of plots. The number of replications required for a certain degree of accuracy has also been worked out in elaborate detail on the basis of these trials. The number of replications required for any experiment depends on the variation between plot to plot in the particular area selected for the experiment and also the degree of accuracy expected in the results. As these two factors are likely to change from field to field, the value of the findings from a particular uniformity trial regarding replications is not of any general use. But the trend shown by the studies on the plot size and the efficiency of different experimental designs is of immense value in improving the technique of field experiments.

The purpose of the present paper is to examine a wheat uniformity trial data with a view to obtain information on a number of points which will be of great use in laying out field experiments. The literature on the different aspects dealt with in this paper will be reviewed in brief when we discuss the respective results.

### MATERIAL

The data of the present investigations were collected at the Agricultural Sub-station, Karnal, in April 1937, at the time of harvesting wheat I. P. 114 from the General Area No. 2 (Plot No. 38). The plot was sown on 11 November 1936 with a Monarch drill with 9-in. spacing between rows, and the crop was irrigated thrice before harvest. There were 0.74 in. of rain in May, 14.33 in. in June, 9.04 in. in July, 6.07 in. in August, 4.85 in. in September, 1.60 in. in December, 0.02 in. in January, 4.68 in. in February, 0.12 in. in March and 1.63 in. in April. The cropping, manuring,

cultural and other operations were uniform over the entire area. The previous cropping history was as follows :—

	<i>Kharif</i> (Monsoon)	<i>Rabi</i> (Winter)
1935-36 . . . . .	Fallow	Wheat I. P. 114
1936-37 . . . . .	Fallow	Wheat I. P. 114

A net area of 400 ft.  $\times$  125 ft. (1.1478 acres) was harvested by dividing it into 80  $\times$  25 (or two thousand 5 ft.  $\times$  5 ft.) units after eliminating a minimum border of 3.5 ft. all round the net area. All the plots were harvested and threshed individually. The outturn of every plot was recorded in ounces after thoroughly drying the grains in the sun. The yields are given in the Appendix.

#### DISTRIBUTION OF PLOT YIELDS

Before taking the distribution of yields for the whole area, the homogeneity of the field was examined by dividing it into four parts or sets as indicated below :

Portion included in set 1—1 to 25 rows and 1-20 columns

Portion included in set 2—1 to 25 rows and 21-40 columns

Portion included in set 3—1 to 25 rows and 41-60 columns

Portion included in set 4—1 to 25 rows and 61-80 columns

The mean yield and its variance for the ultimate plot size 5 ft.  $\times$  5 ft. are given in Table I for the four sets.

TABLE I  
*Mean and variance for the different sets*

Sets	Mean (ounces)	Variance
1 .	17.198	8.808
2 .	15.498	7.875
3 .	16.066	8.675
4 .	17.818	9.570

Table I shows that the whole field cannot be considered as a homogeneous unit. However, there is justification to take sets 2 and 3 together, and 1 and 4 together.

The distribution of yields for a few plot sizes of sets 1, 2, 3 and 4 separately and together have been investigated by finding  $\beta_1$ ,  $\beta_2$ ,  $\chi^2$ , and  $P$  ( $\chi^2$ ) for normal distribution. Sets 1, 2, 3 and 4 have been taken together to see the changes in the values of  $\beta_1$  and  $\beta_2$  for varying plot sizes when the data are not homogeneous. Sets 2 and 3 are taken together to note the variations in  $\beta_1$  and  $\beta_2$  for different plot sizes when the data are homogeneous.

TABLE II

*Distribution constants of yields for a few plot sizes*

Description of plot size		$\beta_1$	$\beta_2$	$\chi^2$	$P(\chi^2)$
Set 1 .	5 ft. $\times$ 5 ft.	$0.186 \pm 0.075$	$2.805 \pm 0.202$	44.4	0.01
	10 ft. $\times$ 5 ft.	$0.169 \pm 0.084$	$2.461 \pm 0.185$	19.1	$<0.05$ and $>0.01$
	10 ft. $\times$ 10 ft.	$0.061 \pm 0.060$	$1.980 \pm 0.149$	13.9	$>0.50$ and $<0.70$
Set 2 .	5 ft. $\times$ 5 ft.	$0.144 \pm 0.080$	$3.074 \pm 0.295$	23.5	$>0.01$ and $<0.05$
	10 ft. $\times$ 5 ft.	$0.023 \pm 0.020$	$2.580 \pm 0.183$	13.0	$>0.20$ and $<0.30$
	10 ft. $\times$ 10 ft.	$0.197 \pm 0.137$	$2.544 \pm 0.301$	22.6	$>0.05$ and $<0.10$
Set 3 .	5 ft. $\times$ 5 ft.	$0.300 \pm 0.153$	$3.512 \pm 0.558$	22.1	$>0.05$ and $<0.10$
	10 ft. $\times$ 5 ft.	$0.162 \pm 0.105$	$2.859 \pm 0.313$	17.7	$<0.05$ and $>0.01$
	10 ft. $\times$ 10 ft.	$0.099 \pm 0.098$	$2.536 \pm 0.272$	28.4	$<0.05$ and $>0.01$
Set 4 .	5 ft. $\times$ 5 ft.	$0.057 \pm 0.065$	$3.485 \pm 0.519$	31.0	0.01
	10 ft. $\times$ 5 ft.	$0.004 \pm 0.006$	$3.339 \pm 0.572$	9.3	$>0.5$ and $<0.7$
	10 ft. $\times$ 10 ft.	$0.000 \pm 0.000$	$2.496 \pm 0.225$	11.1	$>0.7$ and $<0.8$
Sets 2 & 3 .	5 ft. $\times$ 5 ft.	$0.228 \pm 0.087$	$3.361 \pm 0.316$	45.8	$<0.01$
	10 ft. $\times$ 5 ft.	$0.088 \pm 0.051$	$2.806 \pm 0.194$	25.5	$>0.01$ and $<0.02$
	10 ft. $\times$ 10 ft.	$0.156 \pm 0.087$	$2.569 \pm 0.211$	35.6	$<0.01$
Sets 1, 2, 3 & 4	5 ft. $\times$ 5 ft.	$0.157 \pm 0.045$	$3.163 \pm 0.170$	89.9	$<0.01$
	10 ft. $\times$ 5 ft.	$0.045 \pm 0.016$	$2.763 \pm 0.124$	28.2	$>0.01$ and $<0.02$
	10 ft. $\times$ 10 ft.	$0.031 \pm 0.018$	$2.307 \pm 0.096$	42.9	$<0.01$

The distribution of yields for the three plot sizes 5 ft.  $\times$  5 ft., 10 ft.  $\times$  5 ft. and 10 ft.  $\times$  10 ft. of the sets 2, 3 and 4 can be considered to be normal for all practical purposes. The values of  $\beta_1$  and  $\beta_2$  are not, on the whole, significantly different from 0 and 3 respectively.  $P(\chi^2)$  also, more or less, confirms this conclusion. It may be noted that there is a tendency for the value of  $\beta_2$  to diminish as the size of the plot increases. Set 1 cannot be considered to be normally distributed. Taking sets 2 and 3 together the distribution of yields for the smallest plot size is not normal. Values of  $\beta_1$  and  $\beta_2$  for the other sizes are not significantly different from those for the normal distribution, but the values of  $P(\chi^2)$  show that the departure from the normal curve, if not significant, is on the verge of significance. It is seen that the distribution for the whole area is not normal for any of the plot sizes under consideration. Here also it is interesting to note that  $\beta_1$  and  $\beta_2$  diminish as the size of the plot increases. Briefly summarized the above investigations lead to the following conclusions :

- (i) The distribution of yields from smaller areas is more likely to be normal than from larger areas ;
- (ii) There is a tendency for the value of  $\beta_1$  to approach zero as the size of the plot increases ;
- (iii) The value of  $\beta_2$  though not significantly different from 3 in many cases, decreases as the plot size increases ;
- (iv) The distribution of yields approaches normality as the plot size is increased.

#### OPTIMUM SIZE AND SHAPE OF PLOTS

We have already seen that the difference between the means for the ultimate plot sizes of sets 2 and 3 and also those of sets 1 and 4 are not significantly different from each other. This finding taken along with the values of  $\beta_1$  and  $\beta_2$  for these sets shows that the frequency distribution of the primary data for sets 2 and 3, and 1 and 4 could more or less be considered as belonging to the same universe. This was also confirmed by applying Pearson's [1932]  $\chi^2$  method of testing the probability of two samples belonging to the same universe. The values of  $\chi^2$  for sets 2 and 3, and 1 and 4 were 7.4 and 18.5 respectively with 13 degrees of freedom each. Hence investigations regarding the size and shape of plots were carried out separately for sets 2 and 3 together, 1 and 4 together and also for sets 1, 2, 3 and 4 together.

The yields were computed for a large number of plot sizes, and the coefficient of variation for the different plot sizes, after eliminating for soil heterogeneity on the basis of blocks containing five plots running along rows as well as along columns, are given in Table III. This is also shown graphically in Figs. 1 and 2. In dealing with sets 1 and 4 together, care was taken to see that any block of five plots formed for purposes of eliminating soil heterogeneity did not extend from set 1 to set 4. The reduction in error by increased plot size without any elimination for block effects will be seen from the coefficient of variation before elimination given in Table III. This will be useful in fixing the best size of plot for purposes of yield estimation by sampling before harvest.

TABLE III  
Coefficient of variation for different plot sizes

Plot size (rows × co- lumnns)	Area in sq. ft.	Blocks running along rows							Blocks running along columns				
		C. V. before elimina- tion			C. V. after elimina- tion			Average of sets 1, 2, 3 & 4	C. V. after elimina- tion			Average of sets 1, 2, 3 & 4	
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4		
1 × 1	25	17.2	18.0	18.3	13.6	14.3	13.9	13.9	13.8	13.6	13.7	13.7	
1 × 2	50	14.7	15.7	16.0	12.0	12.5	12.2	11.8	11.2	11.3	11.3	11.8	
2 × 1		14.4	15.6	15.7	11.0	11.8	11.4		12.6	11.9	12.3		
1 × 3	75	14.0	14.4	15.2	11.2	11.3	11.2	10.8	10.5	10.3	10.4	10.2	
3 × 1		13.1	14.6	14.7	9.9	10.8	10.3		10.4	9.4	10.0		
4 × 1	100	12.0	14.0	13.8	9.0	10.4	9.7	10.0	11.2	11.5	11.4	10.8	
1 × 4		13.0	13.1	14.0	10.2	9.1	9.8		9.6	9.4	9.5		
2 × 2		12.6	14.1	14.1	10.2	10.8	10.5		12.1	10.5	11.4		
1 × 5	125		12.5			9.0		9.3		8.8		11.8	
5 × 1		12.0	13.3	13.5	10.6	9.8	9.3		11.3	12.5	11.8		
2 × 3	150	12.0	12.9	13.5	9.4	9.6	9.5	9.3	11.8	9.8	10.9	10.6	
3 × 2		11.6	13.3	13.4	9.3	9.8	9.6		12.1	11.5	11.9		
1 × 6			12.5			9.6			8.8	9.0	8.9		
6 × 1		11.2	13.0	13.1	8.4	9.5	8.9						
1 × 7	175		12.4			9.9		8.5	8.7	8.7	8.7	8.7	
7 × 1		11.2	11.8	13.0	8.4	8.6	8.5						
8 × 1	200	10.6	12.1	12.3	7.8	9.4	8.6	8.7				9.6	
1 × 8									8.4	7.8	8.2		
2 × 4		11.3	11.9	12.5	8.9	7.6	8.4		11.1	9.1	10.2		
4 × 2		10.6	12.8	12.6	8.4	9.7	9.0		10.2	10.6	10.4		
3 × 3	225	11.0	12.5	12.8	9.0	8.7	8.9	8.8	11.5	11.2	11.4	9.7	
9 × 1		9.3	10.7	10.7	8.4	9.2	8.8						
1 × 9									8.1	8.1	8.1		
10 × 1	250	8.6	10.6	10.3	7.8	9.0	8.4	8.7				9.3	
1 × 10									7.8	7.4	7.6		
5 × 2		10.9	12.0	12.5	8.9	9.0	9.0		10.4	11.6	11.0		
2 × 5	300		11.0			7.7		8.1	9.6	8.8	9.2	9.3	
2 × 6			11.2			8.4							
6 × 2		10.1	11.8	12.1	8.3	8.5	8.4		9.5	8.8	9.2		
3 × 4		10.5	11.3	12.0	8.2	7.0	7.7		7.7	10.0	8.8		
4 × 3		9.6	12.0	12.0	7.9	8.6	8.2		9.7	10.4	10.0		
7 × 2	350	10.1	10.6	12.0	8.3	7.8	8.1	9.1				8.2	
2 × 7			11.5	11.9		8.6	10.1		9.4	9.0	8.2		
3 × 5	375		10.4			7.1		8.2	7.6	6.7	7.2	8.8	
5 × 3		10.2	11.2	12.0	8.0	8.4	8.2		10.0	11.1	10.5		
2 × 8	400		10.7			8.0		6.6	9.1	7.5	8.8	8.9	
8 × 2		9.6	10.9	11.3	7.6	8.4	5.9						
4 × 4		9.7	10.8	11.3	7.6	6.7	7.2		9.4	9.8	9.6		
9 × 2	450	7.1	9.4	9.8	7.0	7.4	7.2	7.7				8.1	
2 × 9									9.0	7.8	8.5		
8 × 6				11.6			8.2				7.8		
3 × 3		9.8	11.1	11.7	7.6	7.8	7.7						
2 × 10	500			11.5			9.7	8.1			7.9	8.8	
10 × 2				9.0			6.9						
4 × 5				10.8			8.5				8.6		
5 × 4				11.2			7.2				10.0		
3 × 7	525			11.3			9.6	9.6			10.3	8.9	
7 × 3											7.5		
2 × 11	550			10.7			8.5	7.8			9.1	9.1	
11 × 2				9.0			7.1						

TABLE III—*contd*

Plot size (rows × columns)	Area in sq. ft.	Blocks running along rows						Blocks running along columns			
		C. V. before elimination			C. V. after elimination			C. V. after elimination			Average of sets 1, 2, 3 & 4
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	
4 × 6 6 × 4 2 × 12 12 × 2 3 × 8 8 × 3	600		8.7	10.9 10.4 10.1 10.8 10.6		4.5 7.5	6.7 8.1 7.1 9.6 7.2			9.4 8.1 6.6	8.0
		8.8	9.9		6.9						
5 × 5 2 × 13 9 × 3 4 × 7 6 × 5 2 × 16 3 × 11 4 × 10	625 650 675 700 750 800 825 1000			10.6 10.4 8.5 11.0 10.4 9.9 11.1 8.2			8.7 8.1 7.0 9.2 8.1 9.2 9.0 7.6			9.8 7.6 9.3 7.2 10.6 8.6	9.8 7.6 9.3 7.2 10.6 8.6
6 × 7 3 × 14	1050			10.4 10.2			8.9 8.7			10.9	10.9
4 × 13	1300			9.8			7.4			8.7	8.7
18 × 3 9 × 6	1350			6.8 7.7			5.9 6.3				
4 × 14 8 × 7	1400			10.1 10.3			8.5 8.5			8.8	8.8
6 × 10 12 × 5	1500			10.3 8.4			8.3 6.7				
9 × 7	1575			10.7			9.0				
4 × 16 2 × 32	1600			9.1			8.3			6.2 6.4	6.3
6 × 11 6 × 13 8 × 10 9 × 9	1650 1950 2000 2025			10.1 9.6 9.7 6.6			7.7 7.3 7.4 6.5				
6 × 14 12 × 7	2100			9.5 8.6			7.9 8.0				
6 × 15 6 × 16 9 × 11 10 × 10 8 × 13	2250 2400 2475 2500 2600			9.2 9.0 10.9 7.2 8.9			8.2 8.1 7.9 7.6 6.4				
18 × 6 9 × 12	2700			5.8 6.3			5.0 6.7				
8 × 14 12 × 10 9 × 14 8 × 16 12 × 11 12 × 13	2800 3000 3150 3200 3300 3900			9.5 8.1 9.8 8.5 8.2 6.8			7.6 6.3 7.7 7.6 6.1 5.8				

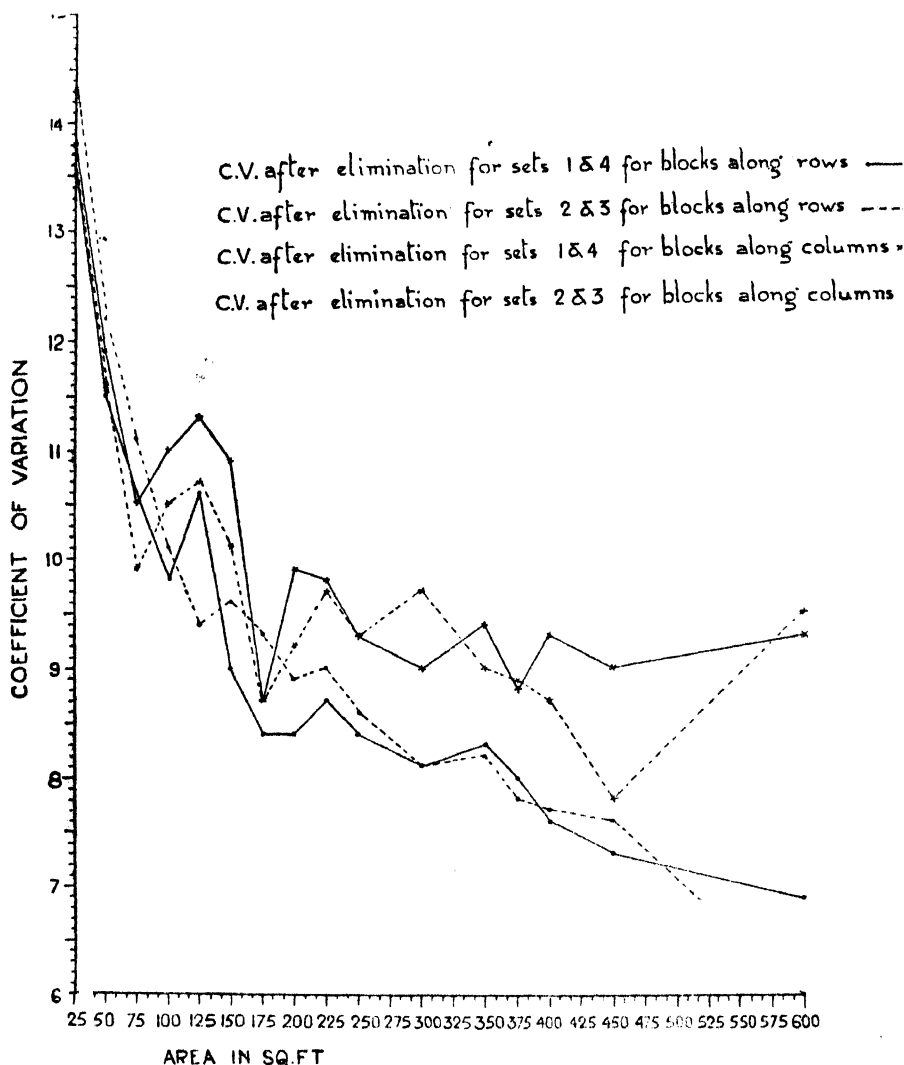


FIG. 1. Average coefficient of variation for different plot sizes

Before discussing Table III we shall make a brief survey of the conclusions arrived at by other workers on the question of size of plots for experiments with wheat. Mercer and Hall [1911] find that the probable error is reduced for all practical purposes to a minimum when the size of each plot is 1/50 acre. Hall and Russell [1911] hold that each treatment should be repeated five times in plots of 1/50 acre in size. Montgomery [1913] concludes that 5 ft.  $\times$  16 ft. is an excellent size when plenty of land is available. Day [1920] finds that the most effective replicated block is the one that is long and narrow and has its greater dimension in the direction of greatest variation. According to Christidis [1931] the plots should be as long and narrow as possible within the limits of practical considerations. Bose [1935] says that the best plot sizes for future experiments appear to be 96 ft.  $\times$  4 ft.

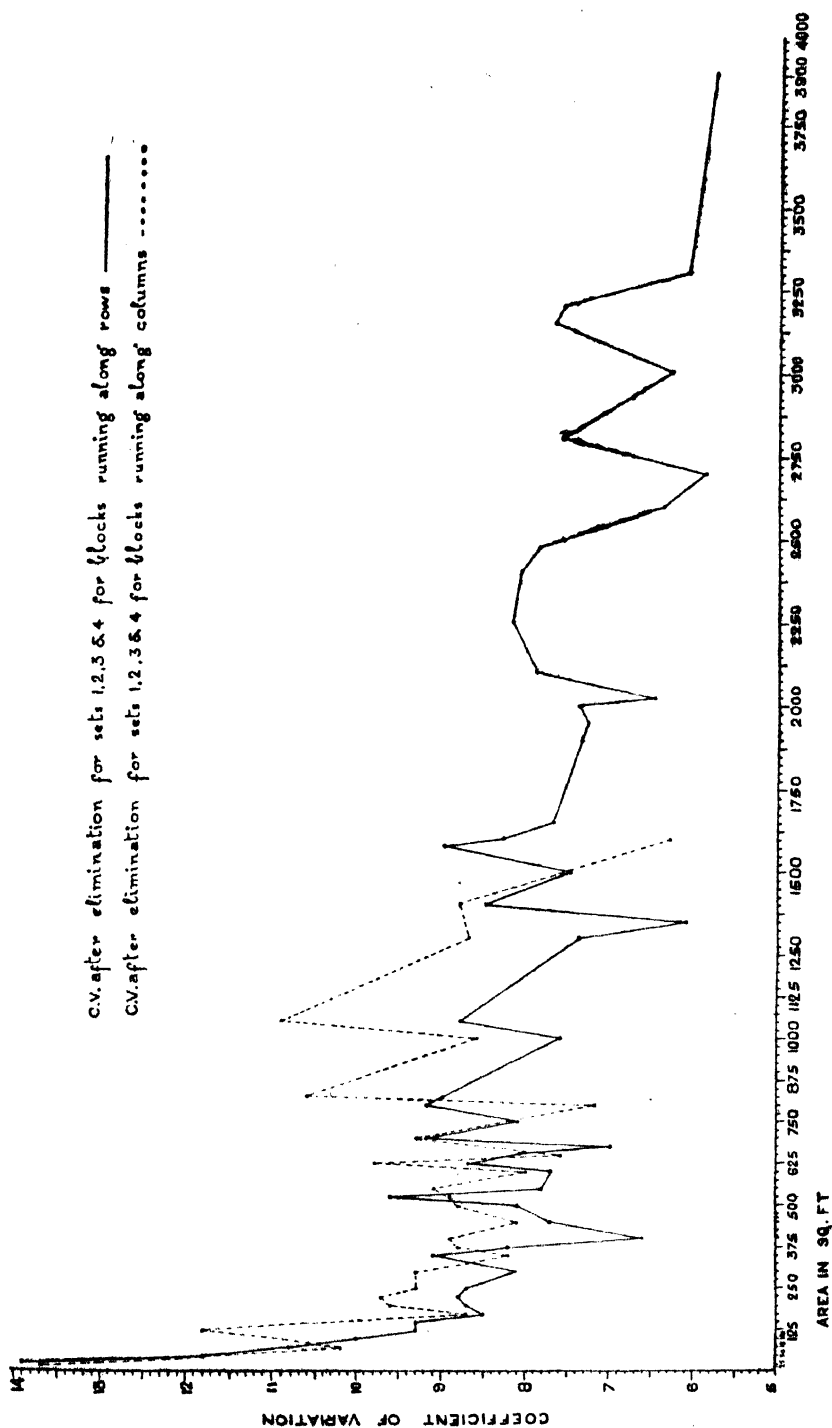


FIG. 2. Average coefficient of variation for different plot sizes

48 ft.  $\times$  12 ft., 32 ft.  $\times$  12 ft. and 24 ft.  $\times$  12 ft. Long and narrow plots running across the fertility trend seem to be preferable to plots which approach a square.

#### *Plot size and C. V.\* before elimination*

The examination of the C. V. before elimination is important for determining the size of sample for estimating yield by sampling. The estimation of yield must be made with as little error as possible and this can be done by fixing a sampling unit with a low error. This information is obtained from the C. V. before elimination for the different areas.

Whether we consider sets 1 and 4 or sets 2 and 3 or all the sets together, the C. V. before elimination for the different plot sizes steadily diminishes with the increase in area up to 225 sq. ft. In the first two cases the C. V. of areas exceeding this size can be considered to be more or less the same with small fluctuations. On taking all the sets together there is further reduction in error when the plot size reaches about 600 sq. ft. and shows still more reduction for areas of 1,600 sq. ft. or more. For areas between 600 sq. ft. and 1,600 sq. ft. the error remains almost the same. As the values for larger plot sizes do not show any consistent trend, much importance cannot be attached to the low errors obtained beyond 1,600 sq. ft. Thus it appears that for estimating yield from small areas, say 5 to 10 acres, the best sampling unit, as judged from this experiment, is about 225 sq. ft. and for larger areas this may be somewhere near 600 sq. ft. or 1600 sq. ft. The area 5 to 10 acres has been fixed on a rough basis on the assumption that the area sampled is about 5 per cent and that the number of sampling units is between 6 and 10.

There is now the question as to the method of collecting this sampling unit. This sample can be taken either from one spot at random or a number of small samples can be taken from different points selected at random and then mixed up to form a composite sample. Commonsense suggests that the latter method might be preferable to the former one. However, it is hoped to deal with this aspect in a subsequent paper by using the same data.

#### *Plot size and C. V. after elimination*

Taking the case of the blocks running along rows the C. V. after elimination is practically a minimum when the area of the plot is about 400 sq. ft. For blocks running along columns also there is considerable reduction in the C. V. with increased plot size up to a certain point, i.e. somewhere about 450 sq. ft. For larger areas, excepting for the largest plot size, the reduction or increase in error is not so marked. It is now clear that the plot size for experiments with wheat can be fixed at about 400 sq. ft.

#### *Shape of plots*

Table IV gives the percentage efficiency ( $100 \times$  ratio of variance before and after elimination) for different plot sizes. Examining this table we find that the elimination for soil heterogeneity is not so effective when the area of the plot is greater than 600 sq. ft. This table also shows that, on the whole, there is greater variation between rows than between columns.

\* C. V. = coefficient of variation

TABLE IV

*Percentage efficiency for different plot sizes*

Plot size (rows × col- umns)	Area in sq. ft.	Blocks running along							
		Rows				Columns			
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4
1 × 1	25	159	159	172	172	155	175	177	177
1 × 2	50	150	158	171	182	174	192	202	176
2 × 1		172	177	192		126	159	150	
1 × 3	75	158	165	184	194	171	197	208	188
3 × 1		174	183	203		136	160	168	
4 × 1	100	178	182	205	197	115	121	143	167
1 × 4		161	206	205		183	196	216	
2 × 2		153	170	182		108	155	142	
1 × 5	125	..	190	..	211	..	208	..	131
5 × 1		182	182	211		113	113	131	
2 × 3	150	164	184	203	205	100	152	139	169
3 × 2		154	182	196		109	167	149	
1 × 6		..	168	..		178	192	218	
6 × 1		177	188	216		..	..	..	
1 × 7	175	..	158	..	232	170	205	221	221
7 × 1		180	185	232		..	..	..	
8 × 1	200	184	165	205	208	..	..	..	172
1 × 8		..	..	..		161	224	236	
2 × 4		160	245	224		103	140	137	
4 × 2		159	173	194		109	115	143	
3 × 3	225	149	207	208	178	97	102	141	189
9 × 1		122	135	147		..	..	..	
1 × 9		..	..	..		172	212	231	
10 × 1	250	122	140	153	173	..	..	..	168
1 × 10		..	..	..		170	224	236	
5 × 2		148	177	192		110	107	129	
2 × 5		..	205	..		108	143	140	

TABLE IV—*contd*

Plot size (rows × columns)	Area in sq. ft.	Blocks running along							
		Rows				Columns			
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4
2 × 6	300	..	176	..	221	104	134	135	135
6 × 2		149	194	208		..	..	..	
3 × 4		161	260	242		147	95	131	
4 × 3		147	198	212		104	104	140	
7 × 2	350	150	187	220	179	..	..	..	208
2 × 7		..	178	138		100	137	208	
3 × 5	375	..	216	..	212	109	116	154	140
5 × 3		162	177	212		103	101	126	
2 × 8	400	..	181	..	304	116	170	184	160
8 × 2		162	168	363		..	..	..	
4 × 4		164	258	244		106	92	135	
9 × 2		104	162	185		..	..	..	
2 × 9	450	..	..	..	205	107	162	177	159
3 × 6		..	..	201		..	..	141	
6 × 3		165	205	228		..	..	..	
2 × 10		..	..	143		..	..	142	
10 × 2	500	..	..	170	178	..	..	..	128
4 × 5		..	..	158		..	..	117	
5 × 4		..	..	239		..	..	124	
3 × 7		..	..	139		..	..	146	
7 × 3	525	..	..	..	139	..	..	232	189
2 × 11	550	..	..	158	158	..	..	128	128
11 × 2		..	..	158		..	..	..	
4 × 6	600	..	372	..	208	87	85	127	137
6 × 4		..	..	265		..	..	..	
2 × 12		..	..	164		..	..	140	
12 × 2		..	..	263		..	..	..	
3 × 8		..	..	129		..	..	144	
8 × 3		162	174	221		..	..	..	
5 × 5	625	..	..	147	147	..	..	121	121
2 × 13	650	..	..	165	165	..	..	194	194
9 × 3	675	..	..	150	150	..	..	..	..
4 × 7	700	..	..	142	142	..	..	138	138
6 × 5	750	..	..	166	166	..	..	..	..
2 × 16	800	..	..	117	117	..	..	130	130
3 × 11	825	..	..	154	154	..	..	133	133
4 × 10	1000	..	..	119	119	..	..	155	155

TABLE IV—*concl'd*

Plot size (rows × columns)	Area in sq. ft.	Blocks running along							
		Rows				Columns			
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4
6 × 7	1050	..	..	136		..	..	..	
3 × 14		..	..	136	136	..	..	134	134
4 × 13	1300	..	..	174	174	..	..	125	125
18 × 3	1350	..	..	130	139	..	..	..	..
9 × 6		..	..	148		..	..	..	
4 × 14	1400	..	..	140		..	..	131	
8 × 7		..	..	147	144	..	..	..	131
6 × 10	1500	..	..	155	155	..	..	..	..
12 × 5		..	..	154		..	..	..	
9 × 7	1575	..	..	142	142	..	..	..	..
4 × 16	1600	..	..	121	121	..	..	127	123
2 × 32		..	..	..		..	..	118	
6 × 11	1650	..	..	175	175	..	..	..	..
6 × 13	1950	..	..	175	175	..	..	..	..
8 × 10	2000	..	..	173	173	..	..	..	..
9 × 9	2025	..	..	104	104	..	..	..	..
6 × 14	2100	..	..	145	131	..	..	..	..
12 × 7		..	..	116		..	..	..	..
6 × 15	2250	..	..	125	125	..	..	..	..
6 × 16	2400	..	..	123	123	..	..	..	..
9 × 11	2475	..	..	192	192	..	..	..	..
10 × 10	2500	..	..	90	90	..	..	..	..
8 × 13	2600	..	..	195	195	..	..	..	..
18 × 6	2700	..	..	136	113	..	..	..	..
9 × 12		..	..	89		..	..		..
8 × 14	2800	..	..	155	155	..	..		..
12 × 10	3000	..	..	164	164	..	..	..	..
9 × 14	3150	..	..	164	164	..	..	..	..
8 × 16	3200	..	..	125	125	..	..	..	..
10 × 11	3300	..	..	181	181	..	..	..	..
12 × 13	3900	..	..	136	136	..	..	..	..

Taking now the plot size 5 ft.  $\times$  10 ft. we note from Table IV that there is greater variation between columns (i.e. across rows) than between rows—thus indicating that there is greater reduction in variability when the length of the plot runs along rows, i.e. in the direction of greater variation. The above fact is borne out by a large number of other plot sizes also. Thus, for all the sizes 10 ft.  $\times$  5 ft., 5 ft.  $\times$  15 ft., 15 ft.  $\times$  5 ft., 20 ft.  $\times$  5 ft., 5 ft.  $\times$  20 ft., 15 ft.  $\times$  10 ft., 5 ft.  $\times$  30 ft., 5 ft.  $\times$  35 ft., 20 ft.  $\times$  10 ft., 25 ft.  $\times$  10 ft., 20 ft.  $\times$  15 ft. and 25 ft.  $\times$  15 ft., the length will be found to run along the direction of greater variation. This finding is in accordance with those of Day [1920] and of Bose [1935]. Day finds that the variability in oblong plots is smaller than in the square ones, provided the length of the plot lies along the direction of greater change of soil fertility. Bose also finds that plots taken at right angles to the direction of the fertility gradient would probably give a smaller variability than those lying along this direction.

Table V shows the relation between L/B and the C. V. after elimination. It will be seen that this table does not lead us to any definite conclusion regarding the relationship between L/B and the C. V. for different plot sizes. The evidence available is not sufficient to enable us to assume any relationship between the C. V. after elimination and L/B. We have already found that, if and when L/B is greater than 1, the length of the plot must run along the direction of greater variation.

TABLE V

*C. V. after elimination for different values of L/B and areas*

L/B	Dimensions of plot	C. V. after elimination	L/B	Dimensions of plot	C. V. after elimination
1.00	5 ft. $\times$ 5 ft.	13.9	1.25	20 ft. $\times$ 25 ft.	8.5
	10 ft. $\times$ 10 ft.	10.5		25 ft. $\times$ 20 ft.	7.2
	15 ft. $\times$ 15 ft.	8.9		40 ft. $\times$ 50 ft.	7.4
	20 ft. $\times$ 20 ft.	7.2	1.29	45 ft. $\times$ 35 ft.	9.0
	25 ft. $\times$ 25 ft.	8.7		15 ft. $\times$ 20 ft.	7.7
	45 ft. $\times$ 45 ft.	6.5	1.33	20 ft. $\times$ 15 ft.	8.2
1.08	50 ft. $\times$ 50 ft.	7.6		45 ft. $\times$ 60 ft.	6.7
	60 ft. $\times$ 65 ft.	5.8	1.50	10 ft. $\times$ 15 ft.	9.5
1.09	60 ft. $\times$ 55 ft.	6.1		15 ft. $\times$ 10 ft.	9.6
	40 ft. $\times$ 35 ft.	8.5		30 ft. $\times$ 20 ft.	6.7
1.14	30 ft. $\times$ 35 ft.	8.9		45 ft. $\times$ 30 ft.	6.3
	30 ft. $\times$ 25 ft.	8.1	1.56	45 ft. $\times$ 70 ft.	7.7
1.20	60 ft. $\times$ 50 ft.	6.3		40 ft. $\times$ 65 ft.	6.4
1.22	45 ft. $\times$ 55 ft.	7.9	1.625	25 ft. $\times$ 15 ft.	8.2
				30 ft. $\times$ 50 ft.	8.3

TABLE V—*contd.*

L/B	Dimensions of plot	C. V. after elimination	L/B	Dimensions of plot	C. V. after elimination
1.71	60 ft. × 35 ft.	8.0	3.50	35 ft. × 10 ft.	8.1
1.75	20 ft. × 35 ft.	9.2		10 ft. × 35 ft.	10.1
	40 ft. × 70 ft.	7.6		20 ft. × 70 ft.	8.5
1.83	30 ft. × 55 ft.	7.7	3.67	15 ft. × 55 ft.	9.0
2.00	5 ft. × 10 ft.	12.2	4.00	20 ft. × 5 ft.	9.7
	10 ft. × 5 ft.	11.4		5 ft. × 20 ft.	9.8
	10 ft. × 20 ft.	8.4		40 ft. × 10 ft.	5.9
	20 ft. × 10 ft.	9.0		20 ft. × 80 ft.	8.3
	15 ft. × 30 ft.	8.2	4.50	45 ft. × 10 ft.	7.2
	30 ft. × 15 ft.	7.7	4.67	15 ft. × 70 ft.	8.7
	40 ft. × 80 ft.	7.6			
2.17	30 ft. × 65 ft.	7.3		25 ft. × 5 ft.	9.3
2.33	15 ft. × 35 ft.	9.6		10 ft. × 50 ft.	9.6
	30 ft. × 70 ft.	7.9		50 ft. × 10 ft.	6.9
2.40	60 ft. × 25 ft.	6.7	5.50	10 ft. × 55 ft.	8.5
2.50	25 ft. × 10 ft.	9.0		55 ft. × 10 ft.	7.1
	20 ft. × 50 ft.	7.6	6.00	30 ft. × 5 ft.	8.9
	30 ft. × 75 ft.	8.2		10 ft. × 60 ft.	8.1
				60 ft. × 10 ft.	7.1
2.67	15 ft. × 40 ft.	9.6		90 ft. × 15 ft.	5.9
	40 ft. × 15 ft.	7.2	6.50	10 ft. × 65 ft.	8.1
	30 ft. × 80 ft.	8.1	7.00	35 ft. × 5 ft.	8.5
3.00	5 ft. × 15 ft.	11.2	8.00	40 ft. × 5 ft.	8.6
	15 ft. × 5 ft.	10.3		10 ft. × 80 ft.	9.2
	30 ft. × 10 ft.	8.4	9.00	45 ft. × 5 ft.	8.8
	45 ft. × 15 ft.	7.0			
	90 ft. × 30 ft.	5.0			
2.00	20 ft. × 65 ft.	7.4	10.00	50 ft. × 5 ft.	8.4

## RELATIVE EFFICIENCY OF DIFFERENT LAY-OUTS

*Randomized blocks versus Latin square*

Doubts have been expressed as to whether Latin squares will really give a smaller residual error than randomized blocks. Neyman *et al.* [1935] find that 'when the size of the Latin square is increased, cases when randomized blocks are more efficient are surprisingly frequent'. 'In some cases when it is not so', they say 'this is due to wrong arrangement of the

randomized blocks'. Yates [1935], on the other hand, after analysing the experiments laid down at Rothamsted, finds that the efficiency of the Latin square is definitely more than that of randomized blocks. Sayer, Vaidyanathan and Iyer [1936] working with sugarcane found that Latin square is more efficient than randomized blocks, except when the latter is provided with sufficient number of replications. In the case of cotton, Hutchinson and Panse [1935] found that 'if there is sufficient knowledge to design the blocks in the most advantageous manner, randomized block can give as efficient a lay-out as Latin square.'

Table VI gives the percentage efficiencies ( $100 \times$  variance before elimination/variance after elimination) for Latin square and randomized block layouts with five treatments formed from the uniformity trial data for a number of plot sizes. The percentage efficiency for blocks running along columns and rows has been taken from Table IV. For Latin squares this has been calculated by taking the ratio of the variance before elimination to the pooled residual variance of the different squares that can be formed for the particular plot size, after eliminating for the effects of rows and columns for each square.

TABLE VI

*Percentage efficiency of Latin square and randomized block arrangements*

Rows $\times$ columns	Area	Percentage efficiency		
		Latin square	Blocks along rows	Blocks along columns
1 $\times$ 1	5 ft. $\times$ 5 ft.	250	172	177
1 $\times$ 2	5 ft. $\times$ 10 ft.	298	171	202
2 $\times$ 1	10 ft. $\times$ 5 ft.	275	192	150
1 $\times$ 3	5 ft. $\times$ 15 ft.	317	184	208
3 $\times$ 1	15 ft. $\times$ 5 ft.	336	203	168
4 $\times$ 1	20 ft. $\times$ 5 ft.	364	205	143
1 $\times$ 4	5 ft. $\times$ 20 ft.	359	205	216
2 $\times$ 2	10 ft. $\times$ 10 ft.	251	182	142
5 $\times$ 1	25 ft. $\times$ 5 ft.	250	211	131
4 $\times$ 2	20 ft. $\times$ 10 ft.	349	194	143
3 $\times$ 3	15 ft. $\times$ 15 ft.	297	208	141

Table VI shows that Latin square is more efficient than randomized blocks. It is of course realized that the usual limitations of the small number of possible arrangements for the optimum plot sizes makes it necessary to be satisfied by comparing the relative efficiencies of the designs with the above plot sizes.

From the above discussions it follows, as has been mentioned by Fisher and Eden [1929], that if it were possible to know beforehand that the soil heterogeneity is only in one direction, randomized blocks would be more efficient than Latin square. But it is very rarely that we know anything about the variation that is likely to occur in the field. Even in cases where uniformity trial has been conducted, it is difficult to predict the direction of the fertility trend. Further, in majority of cases Latin square has been found to be more efficient than randomized blocks and hence Latin square is likely to prove to be more efficient than randomized blocks.

#### *Number of treatments per block*

In randomized block experiments, the elimination for soil heterogeneity ceases to be effective when the size of the block becomes very large. The question as to the number of plots that can be included in any block of such an experiment in order to have effective elimination for soil heterogeneity is considered for a number of plot sizes in Table VII. Blocks consisting of 4, 5, 6, 7, 8, 10, 11, 13, 15, 16 and 20 plots have been taken along rows and the percentage efficiency for different plot sizes has been given in Table VII.

TABLE VII

*Percentage relative efficiency for different block sizes—blocks running along rows*

Rows × columns	Area	Number of treatments per block										
		4	5	6	7	8	10	11	13	15	16	20
3 × 4	15 ft. × 20 ft.	225	242	154	200	185	129	181	151	157	159	116
4 × 4	20 ft. × 20 ft.	206	244	156	204	185	131	183	147	153	156	118
5 × 4	25 ft. × 20 ft.	219	239	143	200	178	122	157	116	148	150	109
6 × 4	30 ft. × 20 ft.	257	265	158	241	203	127	190	168	177	178	117
5 × 5	25 ft. × 25 ft.	270	147	209	131	122	149	143	149	114	110	...
7 × 4	35 ft. × 20 ft.	238	285	164	335	249	130	221	184	195	197	122
6 × 5	30 ft. × 25 ft.	308	166	242	141	128	175	172	179	126	119	...
7 × 5	35 ft. × 25 ft.	330	190	338	151	131	190	185	194	130	123	...
3 × 5	40 ft. × 25 ft.	355	176	250	147	137	169	166	172	131	124	...
4 × 7	30 ft. × 35 ft.	245	136	191	179	174	141	120	...	...	...	...
5 × 6	45 ft. × 30 ft.	121	148	101	111	98	95	96	97	...	...	...
12 × 5	60 ft. × 25 ft.	472	154	249	122	113	160	174	173	120	115	...

Table VII shows that the number of treatments that can be arranged in a block may not exceed 13, the criterion being that the percentage efficiency, barring a few exceptions, continues to be fairly high when the number of treatments ranges from 4 to 13.

*Balanced incomplete and complete randomized blocks*

The method of balanced incomplete randomized blocks designed by Yates [1936] can be used for experiments involving a large number of treatments or varieties. Goulden [1937] comparing the efficiency of this type of lay-out with ordinary randomized blocks concludes that, in general, incomplete block method will give increased efficiency, which is partially correlated with soil heterogeneity. He further says that if the field is very uniform, there may be loss of efficiency. But this is rather unlikely to occur on the average field.

The percentage efficiencies of the incomplete block method compared to the ordinary randomized blocks have been calculated on the basis of certain areas selected from the uniformity trial by using the formula  $\frac{100 t (k-1) s_b^2}{k (t-1) s_i^2}$  for five cases, and are given in Table VIII. In the above formula  $t$ ,  $k$ ,  $s_b^2$  and  $s_i^2$  stand for the number of treatments, the number of plots per blocks, and the residual variances for the complete and incomplete randomized designs respectively.

TABLE VIII

*Percentage efficiencies of balanced incomplete randomized blocks*

Rows × columns	Area	Area selected for examination		No. of treat- ments	No. of replica- tions	No. of treat- ments in each block	No. of times a pair of treat- ments occurs in the whole exper- iment	No. of blocks taken	Efficiency
		Rows	Columns						
1 × 1	5 ft. × 5 ft.	8—18	21—25	11	5	5	2	11	114
3 × 3	15 ft. × 15 ft.	7—21	21—53	11	5	5	2	11	83
5 × 3	25 ft. × 15 ft.	6—25	21—59	13	4	4	1	13	57
6 × 4	30 ft. × 20 ft.	1—24	5—56	13	4	4	1	13	186
4 × 2	20 ft. × 10 ft.	1—24	25—56	16	6	6	2	16	119

The percentage efficiencies for the different values of  $\lambda$ ,  $t$  and  $k$  dealt with in Table VIII have also been calculated on the basis of the results presented in Table VII by assuming  $s_b^2/s_i^2$  to be equal to the ratio of the efficiencies of blocks having  $k$  and  $t$  treatments and taking the product of this ratio with  $\frac{100t(k-1)}{k(t-1)}$ . The results obtained for the three different cases

dealt with in Table VIII are given in Table IX.

Tables VIII and IX show that the percentage efficiency is comparatively greater for the case of 16 treatments than that for 13 or 11. Thus it appears that when the number of treatments to be experimented with is greater than 13, the balanced incomplete randomized block is preferable to the ordinary randomized block.

TABLE IX

*Percentage efficiency of incomplete randomized blocks*

Rows $\times$ columns	$\lambda = 2, t = 16, k = 6$	$\lambda = 2, t = 11, k = 5$	$\lambda = 1, t = 13, k = 4$
3 $\times$ 4 . . . .	86	118	121
4 $\times$ 4 . . . .	89	117	114
5 $\times$ 4 . . . .	85	134	123
6 $\times$ 4 . . . .	79	123	99
5 $\times$ 5 . . . .	169	90	118
7 $\times$ 4 . . . .	74	113	84
6 $\times$ 5 . . . .	181	85	112
7 $\times$ 5 . . . .	244	90	111
8 $\times$ 5 . . . .	179	93	134
6 $\times$ 7 . . . .	..	100	81
9 $\times$ 6 . . . .	..	136	..
12 $\times$ 5 . . . .	192	78	177

## SUMMARY AND CONCLUSIONS

A wheat uniformity trial consisting of two thousand plots each of size 5 ft.  $\times$  5 ft. has been examined to obtain information on the distribution of yields for different plot sizes from different areas, the size and shape of plots, and the comparative efficiencies of 5  $\times$  5 Latin squares and randomized blocks

with five treatments in each block and also the relative efficiencies of randomized blocks having four, five, six, seven, etc. treatments per block. The efficiency of the balanced incomplete randomized blocks has also been compared with the usual randomized blocks for three cases involving 11, 13 and 16 treatments.

The following are the conclusions from the present investigation :—

(i) The distribution of yields from smaller areas is more likely to be normal than from larger areas. The value of  $\beta_1$  approaches zero as the size of the plot increases.  $\beta_2$  is not significantly different from 3 but generally decreases as the plot size increases. The distribution of yields approaches normality as the plot-size is increased.

(ii) For the purpose of estimating yield by sampling from fairly small areas extending from five to ten acres, the best size of the sampling unit appears to be 225 sq. ft. For larger areas, this may be increased from 600 sq. ft. to 1,600 sq. ft. The best size of plot for experiments with wheat is about 400 sq. ft. As regards the shape of plots, no relation seems to exist between L/B and the error for different plot sizes. When L/B is greater than 1, the length of the plot must run along the direction of greater variation.

(iii) In general, Latin square appears to be more efficient than randomized blocks.

(iv) The maximum number of treatments that can be arranged in a single block of a randomized block arrangement in order to have effective elimination for soil heterogeneity can be taken to be about 13. There appears to be no need to have balanced incomplete block design when the number of treatments to be experimented with is 13 or less.

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## APPENDIX

*Uniformity trial at Karnal with Pusa wheat No. 114, 1936-37*

(General Area, Plot No. 38; Area of unit plot = 5 ft. x 5 ft., i.e. 25 sq. ft. Yield of grain in ounces)

Col. Row No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
25	17.5	18.5	18.0	18.0	17.0	20.0	18.5	17.0	14.5	14.0	14.0	15.5	14.5	20.5	15.0	15.5	12.0	13.5	16.5	12.0
24	16.5	14.5	14.0	15.0	17.0	17.0	17.0	18.5	14.5	17.0	15.0	13.5	15.5	17.5	16.5	22.0	15.5	17.5	14.0	15.5
23	13.0	15.5	15.0	15.0	14.5	17.5	17.0	16.5	15.5	15.0	16.5	17.0	14.0	17.0	14.5	19.0	15.0	15.5	15.0	12.0
22	14.0	16.0	15.0	15.5	14.5	12.5	14.5	18.0	17.0	14.5	20.0	19.5	16.0	15.5	14.5	13.5	12.0	14.0	14.0	12.5
21	15.0	15.0	16.0	17.0	16.5	14.0	15.5	16.0	18.5	16.5	15.0	14.0	16.0	13.0	16.0	14.0	15.5	13.0	14.0	14.5
20	15.0	19.0	16.0	15.5	15.0	15.5	15.5	13.0	14.5	18.0	14.5	13.5	13.5	18.0	14.5	16.5	14.0	14.5	14.0	18.0
19	14.5	17.5	18.0	17.0	19.5	16.0	18.0	15.5	17.5	16.0	16.0	13.0	15.5	17.0	18.0	15.5	12.5	15.0	15.5	14.5
18	16.0	18.0	16.0	15.0	19.0	18.0	21.5	15.5	21.0	18.5	14.0	15.0	16.5	16.5	18.5	17.0	17.0	14.0	15.0	11.0
17	16.5	19.5	19.0	23.0	21.0	19.0	23.5	21.0	20.5	20.5	16.5	14.5	14.0	13.0	18.0	17.0	19.5	14.5	15.0	16.5
16	16.0	24.0	23.0	19.5	19.5	22.5	22.0	21.5	20.5	24.0	20.5	14.0	16.5	19.5	17.5	27.0	24.0	23.0	22.0	20.0
15	15.5	23.0	18.0	17.5	20.0	21.5	19.0	23.0	22.0	17.0	22.0	18.0	19.0	19.5	18.5	22.5	19.0	21.5	17.0	18.5
14	15.5	20.0	16.0	13.0	24.0	19.5	19.5	22.5	17.0	17.0	20.0	16.5	20.0	19.5	15.5	24.0	22.0	17.5	16.5	17.0
13	17.5	22.0	20.0	18.5	22.0	20.5	26.5	22.0	21.5	20.5	22.5	21.5	18.5	23.0	18.0	24.5	17.5	21.0	17.5	21.0
12	20.0	27.0	18.0	19.5	22.0	21.5	23.5	17.0	17.5	17.0	21.0	18.0	19.0	21.5	19.0	23.5	20.0	19.5	19.0	21.5
11	22.0	16.5	16.5	21.0	20.5	19.5	25.0	21.5	24.0	16.0	17.0	18.5	21.5	23.5	18.5	15.5	18.0	22.0	17.0	18.0
10	18.5	14.0	15.5	20.5	18.0	23.5	20.0	17.5	18.0	15.0	20.5	20.0	19.0	22.0	16.5	21.0	19.5	22.0	17.0	16.5
9	15.5	14.0	15.5	22.0	19.0	20.0	23.5	18.0	18.5	19.0	24.0	19.0	19.5	18.0	15.0	18.0	19.0	16.5	16.0	15.5
8	15.0	16.5	15.0	18.0	16.0	22.0	24.0	18.5	19.5	19.5	19.0	20.0	19.5	19.5	15.0	18.0	15.5	18.0	14.5	14.5
7	14.5	19.0	16.5	19.5	21.5	19.5	21.0	20.0	5.0	20.5	19.5	22.0	23.0	21.0	17.0	22.0	21.0	20.0	17.0	12.0
6	16.0	12.0	14.0	16.5	15.0	15.0	18.0	15.5	18.0	15.0	16.0	15.5	15.5	12.0	12.0	13.0	20.0	18.5	15.5	21.5
5	16.5	16.5	13.5	19.0	17.0	12.0	20.0	16.5	23.0	17.0	16.0	14.5	14.0	18.0	15.5	15.5	13.5	14.5	14.5	16.0
4	15.0	16.5	16.5	18.0	16.0	14.0	17.5	17.0	18.0	14.0	14.5	19.0	19.5	20.0	16.0	13.0	11.0	19.0	15.0	12.0
3	19.5	17.0	17.5	18.0	17.0	15.0	18.5	17.5	18.5	16.0	13.0	18.0	19.5	15.5	19.5	16.0	15.5	14.0	16.5	13.0
2	17.5	17.5	17.0	18.0	21.5	16.0	17.0	16.0	17.0	15.5	16.0	17.5	19.0	18.5	19.5	18.0	15.5	16.0	18.0	16.5
1	24.0	15.5	15.0	15.5	17.5	15.0	15.0	19.5	17.0	16.5	12.5	17.5	14.0	15.5	13.5	13.0	21.5	14.0	13.5	16.0

## APPE. IX—c

Col. No. Row No.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
25	14.0	12.0	13.0	12.0	13.0	12.5	11.5	14.5	15.5	12.5	15.5	15.0	17.0	13.5	13.5	16.5	14.0	19.5	18.5	15.0
24	14.5	13.0	12.0	14.0	9.5	12.5	17.5	17.0	13.0	16.5	13.5	14.0	15.5	14.0	13.0	15.0	16.5	18.0	16.0	15.0
23	10.5	10.5	12.0	15.5	12.5	13.5	13.5	14.0	14.0	17.0	12.0	14.0	13.0	14.5	12.5	13.5	15.0	16.5	13.5	15.5
22	11.5	12.0	15.5	13.0	13.0	12.0	15.0	14.5	12.0	17.0	12.0	12.5	15.0	13.5	11.0	14.5	14.0	18.5	13.0	15.0
21	16.0	12.5	12.0	16.0	15.5	15.5	15.5	13.0	12.0	14.0	12.5	15.5	15.5	16.0	14.0	14.5	15.5	16.0	15.5	16.0
20	18.0	12.5	13.5	15.5	13.0	14.5	15.0	16.5	13.5	13.0	14.0	18.0	10.5	13.0	12.5	11.5	14.0	14.5	14.5	13.5
19	14.5	15.0	15.0	18.5	15.0	14.0	17.0	18.5	18.0	15.5	13.0	14.0	16.0	15.0	14.0	15.0	14.0	14.5	14.5	17.0
18	17.5	15.0	15.5	17.5	16.5	17.0	14.5	17.5	17.0	17.0	14.0	13.5	15.5	14.0	12.0	15.0	14.5	16.0	15.0	16.5
17	17.5	18.0	16.0	18.0	20.0	15.5	19.5	18.5	17.0	20.0	15.0	15.0	15.0	14.5	14.5	17.0	15.0	19.5	17.0	16.0
16	19.0	20.0	17.0	17.5	18.0	15.0	17.5	19.0	20.0	16.5	14.0	17.0	15.0	13.5	16.0	16.0	17.5	14.5	15.0	16.0
15	19.0	17.0	15.0	15.5	16.0	16.0	17.0	21.0	16.5	14.5	16.0	13.0	16.0	15.5	14.5	18.5	20.0	12.5	16.0	17.0
14	20.5	18.0	15.5	17.5	18.0	14.5	13.0	15.5	16.0	14.5	15.5	16.0	13.0	13.0	16.0	20.5	20.5	17.5	12.5	14.0
13	22.0	22.0	21.5	19.5	17.0	12.0	18.0	20.5	23.5	16.0	14.0	13.0	14.0	17.0	19.0	22.0	22.0	25.0	13.5	20.0
12	20.5	18.0	22.5	18.0	15.5	13.0	18.0	18.0	19.0	14.5	15.5	18.5	16.0	19.0	15.5	19.0	19.0	20.0	20.0	15.5
11	16.5	21.0	13.5	17.0	17.0	12.0	15.5	21.0	23.0	22.0	18.5	16.0	14.0	18.5	17.5	19.5	21.5	19.5	14.5	13.5
10	18.5	18.5	12.0	15.5	14.5	12.5	15.5	21.0	19.5	18.0	15.5	14.5	14.5	16.0	18.0	19.0	18.5	21.5	15.0	19.5
9	19.0	21.0	16.0	19.5	16.0	15.0	14.0	14.0	13.5	15.0	12.0	15.0	14.0	15.5	15.5	18.0	15.0	17.0	17.0	19.5
8	10.5	16.0	13.5	15.5	16.0	14.5	17.0	20.5	19.0	13.0	16.0	12.0	12.0	16.0	17.0	15.5	14.0	18.5	16.0	17.0
7	10.5	17.0	14.0	15.0	15.5	15.5	19.5	23.0	19.0	15.0	17.0	15.5	13.0	15.5	12.5	15.5	14.5	13.0	9.5	17.0
6	19.0	20.5	18.0	21.5	21.0	19.0	19.0	20.5	13.0	15.0	18.0	14.5	15.5	16.0	18.0	16.5	16.0	20.0	13.0	16.0
5	19.5	21.5	18.0	17.5	17.5	15.0	19.5	16.5	20.5	22.0	20.5	17.5	17.0	17.0	19.0	20.5	17.0	20.5	15.0	18.0
4	16.0	19.0	16.5	15.5	14.5	15.0	21.0	19.0	14.5	12.0	16.5	14.0	13.0	12.0	14.5	20.0	14.5	17.0	12.0	13.5
3	14.0	15.5	13.5	18.0	15.5	12.0	23.5	18.5	13.0	10.0	13.0	14.5	12.0	14.0	17.0	17.0	15.5	18.5	10.5	16.0
2	16.0	15.0	8.0	12.5	14.5	14.0	24.5	20.5	12.0	10.0	14.0	10.0	11.0	16.0	13.5	14.5	13.5	16.0	13.0	19.5
1	15.5	14.0	9.5	9.0	13.0	10.5	16.5	17.0	13.0	12.0	17.5	19.5	13.0	12.5	14.0	15.5	13.0	16.5	10.5	16.5

## APPENDIX—contd.

Col. No. Row No.	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
25	15.0	12.5	13.5	15.0	13.0	11.5	15.5	16.0	17.0	19.0	20.0	17.5	17.0	17.0	17.5	18.0	16.5	14.0	17.0	16.5
24	14.5	10.0	13.0	13.0	16.5	14.5	10.0	15.0	12.5	15.5	17.0	14.0	15.5	15.0	13.0	12.0	14.5	11.5	16.5	15.5
23	11.5	11.0	14.5	13.0	12.5	17.0	14.0	11.0	14.0	13.0	11.0	15.0	15.0	18.0	14.5	16.0	14.5	14.0	12.5	14.0
22	14.0	11.0	17.0	12.0	14.0	13.0	12.0	15.5	12.5	14.0	13.5	14.0	13.0	15.5	15.5	12.5	13.0	12.0	12.5	14.0
21	13.0	11.0	16.5	14.0	15.5	16.0	14.0	15.0	12.5	15.0	14.0	13.0	17.0	13.0	15.0	14.0	12.5	11.0	15.5	16.0
20	13.5	10.5	15.5	16.0	15.5	13.5	15.5	13.0	15.0	13.5	11.0	12.0	16.5	14.5	14.0	13.5	16.0	13.5	13.5	14.0
19	17.0	13.5	14.0	13.5	16.0	18.5	13.0	17.5	15.0	15.5	11.0	16.0	15.0	13.5	15.5	17.0	15.0	13.0	15.0	20.5
18	16.0	14.0	19.5	13.5	17.0	16.0	13.5	11.0	16.0	15.5	13.5	16.0	16.5	16.5	16.0	16.0	18.0	14.0	19.0	17.0
17	17.0	14.5	17.5	18.0	17.5	18.5	15.5	14.5	14.5	14.0	11.0	16.0	15.0	14.0	15.0	13.5	15.5	17.5	18.0	18.5
16	15.5	14.0	21.0	15.5	18.0	18.5	13.0	12.5	12.5	14.5	14.0	15.0	15.5	15.5	19.0	16.5	16.0	14.5	20.0	17.0
15	16.0	11.0	15.0	17.5	15.0	16.5	13.0	11.5	16.0	13.5	12.5	15.5	16.0	17.0	19.5	15.5	17.0	16.0	17.5	13.0
14	16.5	14.0	18.0	13.5	17.0	13.0	15.0	12.0	13.0	14.5	12.5	11.0	15.0	12.5	16.0	14.5	18.5	15.5	13.5	19.5
13	15.0	14.0	18.0	18.0	20.5	19.0	14.0	14.5	12.0	15.5	12.5	12.0	14.5	18.0	22.0	14.5	19.0	15.0	19.0	19.5
12	22.0	17.5	22.5	18.5	18.5	20.5	15.5	15.0	11.0	13.5	18.5	13.5	17.5	19.0	19.5	19.5	19.5	16.5	16.0	16.0
11	15.5	13.0	14.0	19.5	20.5	19.0	17.0	15.0	17.5	19.5	16.0	15.0	17.5	16.0	17.0	19.5	18.0	16.0	16.0	18.5
10	16.0	15.0	19.5	17.0	17.5	20.5	19.5	17.0	18.5	20.5	19.0	16.5	16.5	16.5	23.5	18.0	18.5	12.5	17.0	20.5
9	18.0	14.0	17.0	20.5	20.5	22.0	13.0	16.5	19.0	16.0	15.5	15.5	16.0	16.5	23.5	15.5	15.5	16.0	19.0	17.5
8	16.0	14.5	20.0	20.0	18.0	16.5	12.5	16.0	15.0	15.0	13.5	14.0	14.5	16.5	23.5	18.5	17.0	15.0	17.0	20.0
7	13.0	13.5	18.5	14.0	16.0	17.0	17.5	15.5	15.5	14.0	14.0	15.0	13.0	19.0	21.0	19.0	18.0	18.5	15.0	17.0
6	15.5	12.0	20.0	18.0	20.5	18.5	17.0	16.0	17.0	14.0	17.5	15.5	17.0	17.0	21.0	19.5	19.0	16.0	19.0	18.0
5	21.0	16.0	23.0	21.0	18.0	21.5	21.5	18.0	22.0	21.5	19.5	15.5	16.5	10.0	30.0	21.0	18.5	15.0	16.0	17.0
4	18.0	14.0	22.5	19.5	20.0	26.5	22.5	20.0	25.5	20.5	20.0	18.0	19.0	17.5	19.0	16.0	17.5	16.0	18.0	14.5
3	16.5	18.0	18.0	17.5	19.5	19.0	17.0	18.5	21.5	22.0	27.0	21.0	18.5	22.5	18.5	15.0	14.0	12.0	17.0	20.5
2	14.0	15.5	21.0	25.0	21.5	21.0	23.0	16.0	21.5	23.0	19.0	22.0	19.0	24.0	19.5	18.5	18.0	15.5	15.5	18.5
1	15.5	15.0	17.5	13.0	17.5	16.0	19.0	16.0	20.0	17.0	19.0	20.0	21.5	17.5	20.0	15.0	14.5	13.5	14.5	16.5

## APPENDIX—concd

Row No.	Col. No.	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
25		14.5	15.5	19.0	22.0	22.0	25.0	27.5	27.5	24.0	21.0	24.0	26.0	19.0	18.5	24.0	21.5	24.5	20.0	20.5	23.0
24		16.5	11.0	15.5	15.5	19.0	20.0	22.0	20.5	21.0	22.5	27.0	21.0	16.5	22.0	19.5	20.0	24.0	25.5	22.5	21.0
23		14.5	14.0	16.5	15.0	14.5	16.5	19.5	18.0	20.0	16.5	17.0	18.0	15.5	18.0	22.0	17.0	18.0	20.5	16.5	17.5
22		12.0	12.5	16.5	16.0	14.5	19.0	21.0	23.5	19.5	15.0	17.5	16.5	15.5	16.5	21.5	24.5	18.0	19.5	17.5	18.0
21		14.5	13.5	15.5	18.5	17.5	20.5	22.0	19.0	24.0	23.5	25.5	19.0	19.0	16.0	17.5	19.5	21.0	23.0	18.0	16.5
20		15.0	15.0	16.5	17.0	16.5	17.0	16.5	18.5	15.5	19.0	23.0	20.5	21.5	18.0	15.5	15.0	15.5	18.0	17.0	16.5
19		14.5	16.5	20.5	18.5	18.0	15.0	20.5	16.5	18.5	19.0	18.5	16.5	18.5	20.5	23.0	20.5	19.5	14.5	15.0	17.0
18		15.5	17.0	20.0	17.5	18.5	19.5	24.0	18.0	15.5	17.0	16.5	14.0	14.0	12.0	19.5	19.0	19.0	21.5	13.5	19.5
17		19.5	18.5	18.5	23.5	22.0	26.0	19.5	22.5	22.5	19.0	17.0	21.0	17.5	15.5	19.5	17.0	16.5	24.0	16.5	16.0
16		15.5	16.0	20.0	21.5	19.0	21.5	19.5	25.5	22.0	20.0	17.0	21.5	21.0	17.0	21.0	20.5	16.5	23.5	20.5	21.0
15		19.5	18.0	17.0	20.5	17.0	21.0	19.5	23.0	19.5	15.5	15.0	15.5	18.5	16.0	18.5	23.5	19.0	18.0	15.5	23.0
14		17.5	18.5	17.0	23.0	20.5	19.5	18.5	18.0	9.5	8.0	8.5	14.0	15.0	17.0	19.0	15.5	19.0	18.0	15.0	18.5
13		15.0	17.5	22.0	21.0	22.5	19.0	18.0	14.0	7.5	14.0	13.0	15.0	14.5	18.5	18.0	16.0	14.5	17.5	15.0	20.5
12		19.5	16.5	18.5	18.5	22.5	23.5	21.0	17.5	10.5	12.5	18.0	16.0	17.5	15.5	20.0	21.0	16.5	19.5	20.0	20.0
11		17.0	19.5	24.5	22.5	20.5	25.0	20.5	18.0	19.5	14.5	16.0	21.0	18.0	16.5	17.5	18.0	17.5	19.0	16.5	21.0
10		15.5	19.0	17.5	19.5	23.0	20.0	18.0	17.5	19.0	16.0	18.0	14.5	15.5	15.5	16.0	16.0	15.0	20.0	18.0	23.5
9		18.0	20.5	19.5	19.0	16.5	18.0	18.5	17.5	14.5	19.5	14.0	18.0	18.0	18.0	16.5	18.0	17.0	20.0	20.0	18.0
8		15.0	15.0	15.5	21.0	18.0	22.5	18.5	19.0	18.5	16.5	15.5	15.0	17.0	20.0	20.0	15.0	18.0	24.5	17.5	21.0
7		18.0	16.5	15.0	16.0	17.0	15.5	18.0	19.5	16.5	18.0	20.0	19.0	19.0	19.5	21.0	18.5	21.0	26.0	17.5	23.5
6		15.5	21.0	17.0	17.5	19.5	19.5	18.0	16.0	15.5	12.0	14.0	12.5	16.5	16.5	15.0	18.0	18.0	26.0	22.0	22.0
5		14.5	20.0	16.0	17.5	18.5	19.0	18.5	19.0	13.0	15.0	14.5	13.5	16.0	17.5	16.0	18.5	16.0	20.5	20.5	21.0
4		17.0	16.0	15.5	16.5	20.0	15.5	18.0	18.0	16.0	13.5	12.5	15.0	15.5	16.5	18.0	17.5	15.5	20.0	20.0	23.5
3		13.5	16.5	16.0	16.0	20.5	20.5	18.5	15.5	13.5	17.0	18.0	17.0	15.0	14.5	17.0	15.0	14.0	16.5	16.5	24.0
2		14.5	19.5	16.0	19.5	24.0	20.0	18.0	16.0	15.5	16.5	16.0	15.5	15.5	17.5	16.0	15.0	14.5	13.0	19.0	22.5
1		14.0	11.0	14.0	18.5	15.5	15.5	15.0	16.0	18.5	14.0	13.0	15.0	13.0	13.0	14.0	16.0	14.0	18.0	18.0	15.5

The above data were collected under the supervision of Mr S. Sen

## II. BALANCED VERSUS RANDOMIZED ARRANGEMENTS

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**I**N a paper entitled 'Co-operation in large scale experiments' Gosset [1936] expressed the opinion that 'the advantages of artificial randomization are usually offset by an increased error when compared to balanced arrangements.' Fisher, who does not agree with this view, carried out some investigations with a uniformity trial data in collaboration with Barbaek [1936] and found that random arrangements would give smaller errors than systematic ones. Gosset [1937] examined this point in detail from the practical and the theoretical points of view and has come to the conclusion that balanced arrangements are likely to be more accurate than random designs. In support of his conclusion he has quoted Hudson who, as a result of some investigations on the uniformity trial data of Mercer and Hall [1911], Kalamkar [1932] and Immer [1932], found the balanced arrangements to be more accurate than the random arrangements. Pearson [1938] applying the power tests evolved by Neyman and himself [1936] to some of the results obtained by Hudson has shown that Gosset's view is likely to be more accurate than that of Fisher. Yates [1938] has pointed out that Hudson's results are of little interest for the following reasons :—

- (i) Only three uniformity trials had been used ;
- (ii) The plots in most of the arrangements were only one or two rows wide ;
- (iii) The arrangements used by Hudson were randomized blocks for random arrangements and Latin squares for balanced arrangements. Such a comparison was not fair in view of the fact that Latin squares are more accurate than randomized blocks.

After some further careful analysis he came to the conclusion that 'in cases where Latin square designs can be used and in many cases where randomized blocks have to be employed, the gain in accuracy with the systematic arrangements is not likely to be sufficiently great as to outweigh the disadvantages to which the systematic designs are subject.'

In order to have a correct idea of the relative merits of the two designs, it is necessary to examine the results that would be obtained from other uniformity trials after taking into consideration some of the many alternative arrangements that can be had for both the designs. In this paper the wheat uniformity trial data discussed in part I, have been used to examine the relative accuracy of random and systematic designs from a more comprehensive

point of view for experiments involving four, five, six and seven treatments with six, six, eight and six replications respectively.

### MATERIAL

The material used in this investigation consists of certain portion of the wheat uniformity trial detailed in Tables I-IV.

TABLE I

*Four treatments with 6 replications each plot—3 rows  $\times$  3 columns*  
(Yields in ounces)

Row No.	Column No.	64—66	67—69	70—72	73—75
19—21 . . . . .		158·5	171·0	184·5	169·5
16—18 . . . . .		189·0	189·0	163·0	157·0
13—15 . . . . .		184·0	147·5	118·5	155·0
10—12 . . . . .		195·0	161·5	146·5	152·0
7—9 . . . . .		163·5	160·5	155·5	169·0
4—6 . . . . .		163·5	152·0	124·5	147·5

TABLE II

*Five treatments with 6 replications each plot—3 rows  $\times$  3 columns*  
(Yields in ounces)

Row No.	Column No.	45—47	48—50	51—53	54—56	57—59
19—21 . . . . .		137·5	132·0	125·5	130·0	125·0
16—18 . . . . .		147·5	125·0	132·5	142·0	152·5
13—15 . . . . .		143·0	122·5	121·5	149·5	153·0
10—12 . . . . .		168·5	147·5	150·0	168·5	150·0
7—9 . . . . .		158·0	142·5	131·0	173·0	151·0
4—6 . . . . .		186·0	174·5	158·5	170·0	155·0

TABLE III

*Six treatments with 8 replications each plot—3 rows  $\times$  3 columns*

(Yields in ounces)

Row No.	Column No.	1—3	4—6	7—9	10—12	13—15	16—18
22—24 . . . . .		133·5	138·5	148·5	148·0	141·0	144·0
19—21 . . . . .		146·0	146·0	144·0	136·5	141·5	130·5
16—18 . . . . .		168·0	176·5	187·0	157·5	150·0	173·0
13—15 . . . . .		167·5	176·5	193·0	175·0	171·5	189·5
10—12 . . . . .		168·0	186·0	184·0	163·0	180·5	181·0
7—9 . . . . .		141·5	177·5	183·0	182·5	175·0	169·0
4—6 . . . . .		136·5	142·5	163·5	141·5	142·5	138·0
1—3 . . . . .		160·5	153·5	156·0	142·5	154·5	143·5

TABLE IV

*Seven treatments with 6 replications each plot—3 rows  $\times$  4 columns*

(Yields in ounces)

Row No.	Column No.	21—24	25—28	29—32	33—36	37—40	41—44	45—48
20—22 . . . . .		168·0	173·0	166·0	161·5	180·0	164·0	172·5
17—19 . . . . .		198·0	203·5	189·0	177·5	189·5	188·0	188·5
14—16 . . . . .		211·5	200·5	189·5	187·5	193·0	187·5	175·0
11—13 . . . . .		232·0	197·5	213·5	211·0	224·0	207·5	209·0
8—10 . . . . .		195·5	190·5	183·0	191·0	208·5	207·5	214·5
5—7 . . . . .		212·0	221·5	212·5	196·0	189·5	205·5	217·0

## METHODS

*Four treatments and six replications*

(i) *Random arrangements.*—Three hundred random arrangements were formed by superposing four hypothetical treatments, A, B, C and D on a random basis on the data presented in Table I by considering the blocks to lie

along rows. It can be easily seen that the sum of squares for treatments *plus* residual error is a constant. The error for the treatments is different for different arrangements and has been calculated from the hypothetical treatment totals computed in the manner described below :—

Twenty-four identical blank cards were divided into six groups, each group having four cards. The figures in the six rows of Table I were entered in the six groups of cards. Each group of cards was then thoroughly shuffled and the dummy treatments A, B, C and D were superposed on the 1st, 2nd, 3rd and 4th cards respectively, for all the six groups of cards. The treatment totals were now got by adding up all the A's, B's, C's and D's. The sum of squares for the treatments and the residual error corresponding to the first random arrangement were calculated on the basis of these totals. The above process was repeated 300 times and the treatment and the residual errors were thus obtained for 300 random samples.

(ii) *Balanced arrangements*.—As in the case of randomized arrangements, there are different ways of balancing any experiment, the only difference being that the number of arrangements that can be formed on a random fashion is much more than that on balanced basis. The treatment and the residual errors have been calculated for the arrangements given below for the case of four treatments :—

A	B	C	D
D	C	B	A
A	B	C	D
D	C	B	A
A	B	C	D
D	C	B	A

Chart 1. (Balancing has been effected between two rows, and the same arrangement of the treatments has been repeated in the third and fifth rows)

(1)				(2)			
A	B	C	D	A	B	C	D
B	C	D	A	D	A	B	C
C	D	A	B	C	D	A	B
D	A	B	C	B	C	D	A
A	B	C	D	A	B	C	D
B	C	D	A	D	A	B	C

Chart 2. (Formed on the basis of the two diagonal squares)

A	D	C	B
B	C	D	A
C	B	A	D
D	A	B	C
A	D	C	B
B	C	D	A

Chart 3. (Formed on the basis of Latin squares by effecting balance between two rows and two columns)

The sum of squares for treatments for any of the designs shown in charts 1, 2 and 3 is independent of their arrangements in the first row so long as the scheme of balancing is as explained in the corresponding designs. If  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$  are the treatment totals for A, B, C and D respectively for a particular arrangement of the treatments in the first row (for any of the designs shown

in charts 1, 2 and 3), their totals for any other permutation of the first row will also be the same, the only difference being that  $T_1$ , instead of being the total for treatment A, will now be the total for some other treatment,  $T_2$  will be the total for either B or some other treatment, and so on. Thus, whatever be the permutation of the treatments in the first row, their totals remain the same but get themselves allotted in different ways. Hence the sum of squares for the dummy treatments is fixed so long as the scheme of balancing is fixed.

In addition to the designs described above, 25 more arrangements were formed by balancing two rows and by having different treatment arrangements in the first, third and fifth rows as indicated in chart 4.

A	B	C	D	}	(a)
D	C	B	A		
D	B	A	C	}	(b)
C	A	B	D		
B	C	D	A	}	(c)
A	D	C	B		

Chart 4. (Balancing effected between two rows with the treatment arrangements in a, b and c at random)

The mean and the variance of the treatment errors (i.e. variance for treatments) of the 25 balanced arrangements have been compared with those of the 300 random ones.

The calculation of the treatment sum of squares for each of the balanced designs of the type shown in chart 4 was done as follows :—

The treatment totals for the design shown in chart 4 are the sum of their respective totals for sections (a), (b) and (c). Let the treatment totals for section (a) be  $t_{1a}$ ,  $t_{2a}$ ,  $t_{3a}$  and  $t_{4a}$  for A, B, C and D respectively. For any other arrangement of the treatments in this section the totals will still be  $t_{1a}$ ,  $t_{2a}$ ,  $t_{3a}$  and  $t_{4a}$  but get allotted to different treatments in accordance with the permutation of the treatments in that section. A similar property holds good for sections (b) and (c) also. In view of this property, the treatment totals corresponding to any arrangement of the type shown in chart 4 can be calculated by taking the sectional totals  $t_{1a}$ ,  $t_{2a}$ ,  $t_{3a}$ ,  $t_{4a}$ ;  $t_{1b}$ ,  $t_{2b}$ ,  $t_{3b}$ ,  $t_{4b}$ ;  $t_{1c}$ ,  $t_{2c}$ ,  $t_{3c}$ ,  $t_{4c}$  and allotting them at random to A, B, C and D in each section and then adding up the A's, B's, C's and D's. This was done by writing down the three sets of totals in three groups of identical cards, each group having four cards. Each of the three groups was thoroughly shuffled and the treatments A, B, C and D were allotted to the first, second, third and fourth cards. The totals of the treatments were now computed and the treatment variance was calculated on the basis of these totals. Such a procedure was repeated 25 times and the variances for treatments corresponding to 25 balanced arrangements of the type shown in chart 4 were calculated.

#### *Five treatments and six replications*

(i) *Randomized arrangements.*—The treatment and the residual errors for 400 random arrangements were calculated on the same lines as described for four treatments.

(ii) *Balanced arrangements*.—The following systematic or balanced arrangements have been compared with the 400 random arrangements mentioned above.

A	B	C	D	E
E	D	C	B	A
A	B	C	D	E
E	D	C	B	A
A	B	C	D	E
E	D	C	B	A

Chart 5. (Formed on the same lines as indicated in chart 1)

(1)					(2)				
A	B	C	D	E	A	B	C	D	E
B	C	D	E	A	E	A	B	C	D
C	D	E	A	B	D	E	A	B	C
D	E	A	B	C	C	D	E	A	B
E	A	B	C	D	B	C	D	E	A
A	B	C	D	E	A	B	C	D	E

Chart 6. (Formed on the same lines as shown in chart 2)

(1)					(2)				
A	B	C	D	E	A	B	C	D	E
D	E	A	B	C	C	D	E	A	B
B	C	D	E	A	E	A	B	C	D
E	A	B	C	D	B	C	D	E	A
C	D	E	A	B	D	E	A	B	C
A	B	C	D	E	A	B	C	D	E

Chart 7. (Formed on the basis of Knut Vik squares)

The mean and the variance of the treatment errors of 25 arrangements balanced on lines similar to that described for four treatments were also calculated.

#### *Six treatments and eight replications*

(i) *Random arrangements*.—Four hundred random arrangements were taken as described before.

(ii) *Balanced arrangements*.—The following systematic or balanced arrangements have been taken in this case.

A	B	C	D	E	F
F	E	D	C	B	A
A	B	C	D	E	F
F	E	D	C	B	A
A	B	C	D	E	F
F	E	D	C	B	A
A	B	C	D	E	F
F	E	D	C	B	A

Chart 8. (Arrangement is similar to chart 1)

(1)						(2)					
A	B	C	D	E	F	A	B	C	D	E	F
B	C	D	E	F	A	F	A	B	C	D	E
C	D	E	F	A	B	E	F	A	B	C	D
D	E	F	A	B	C	D	E	F	A	B	C
E	F	A	B	C	D	C	D	E	F	A	B
F	A	B	C	D	E	B	C	D	E	F	A
A	B	C	D	E	F	A	B	C	D	E	F
B	C	D	E	F	A	F	A	B	C	D	E

Chart 9. (Arrangement based on diagonal squares)

	(1)	(2)	(3)	(4)
<i>a</i>	{ A B C D E F F E D C B A	{ A B C D E F F E D C B A	{ A B C D E F F E D C B A	{ A B C D E F F E D C B A
<i>b</i>	{ C F B E A D D A E B F C	{ B C A F D E E D F A C B	{ B A E F C D D C F E A B	{ C F A B D E E D B A F C
<i>c</i>	{ B C A F D E E D F A C B	{ C F B E A D D A E B F C	{ C F A B D E E D B A F C	{ B A E F C D D C F E A B
<i>d</i>	{ A B C D E F F E D C B A	{ A B C D E F F E D C B A	{ A B C D E F F E D C B A	{ A B C D E F F E D C B A

Chart 10. (Arrangement based on two Latin squares)

Excepting for an interchange between the positions of *b* and *c*, arrangements (1) and (2) are the same. Similar is the case with (3) and (4) also.

In addition to the above, as in other cases, the means and the variances for the treatment errors of 25 arrangements balanced as shown in chart 4 were also calculated.

### Seven treatments and six replications

(i) *Randomized arrangements*.—Here also, the treatment and the residual errors were calculated for 400 random arrangements.

(ii) *Balanced arrangements*.—The following systematic or balanced arrangements have been compared with the 400 random arrangements.

A	B	C	D	E	F	G
G	F	E	D	C	B	A
A	B	C	D	E	F	G
G	F	E	D	C	B	A
A	B	C	D	E	F	G
G	F	E	D	C	B	A

Chart 11. (Arrangement similar to that of chart 1)

(1)							(2)						
A	B	C	D	E	F	G	A	B	C	D	E	F	G
B	C	D	E	F	G	A	G	A	B	C	D	E	F
C	D	E	F	G	A	B	F	G	A	B	C	D	E
D	E	F	G	A	B	C	E	F	G	A	B	C	D
E	F	G	A	B	C	D	D	E	F	G	A	B	C
F	G	A	B	C	D	E	C	D	E	F	G	A	B

Chart 12. (Arranged on the basis of diagonal squares)

(1)							(2)						
A	B	C	D	E	F	G	A	B	C	D	E	F	G
F	G	A	B	C	D	E	C	D	E	F	G	A	B
D	E	F	G	A	B	C	E	F	G	A	B	C	D
B	C	D	E	F	G	A	G	A	B	C	D	E	F
G	A	B	C	D	E	F	B	C	D	E	F	G	A
E	F	G	A	B	C	D	D	E	F	G	A	B	C

Chart 13. (Arranged on the basis of Knut Vik squares)

Like the other three cases, the mean and the variance of the treatment errors were calculated for 25 arrangements of the type shown in chart 4 by the same method as that described for four treatments.

### RESULTS AND DISCUSSIONS

The residual and the treatment errors of the different types of balanced arrangements shown above have been compared with those of the randomized arrangements and the results are given below in the succeeding tables.

Table V gives the numbers of randomized arrangements having greater and less residual variance than that for balanced arrangement of the type shown in chart 1.

TABLE V

*Comparison between randomized arrangements and balanced arrangements shown in chart 1*

No. of treatments	No. of replications	No. of samples taken	No. of random samples with residual variance	
			Greater than chart 1	Less than chart 1
4 . . . . .	6	300	240	60
5 . . . . .	6	400	357	43
6 . . . . .	8	400	238	162
7 . . . . .	6	400	314	86

From Table V it is clear that balanced arrangements of the type indicated in chart 1 are likely to give less residual variance than randomized designs and hence comparisons based on random arrangements are likely to be more reliable than that of chart 1.

Table VI shows the numbers of randomized arrangements with greater as well as with less residual variance as compared with the arrangements based on diagonal squares.

TABLE VI  
*Comparison between random arrangements and diagonal squares*

No. of treatments	No. of random samples with residual variance			
	Greater than chart 2 (1)	Less than chart 2 (1)	Greater than chart 2 (2)	Less than chart 2 (2)
4 . . . . .	199	101	56	244
5 . . . . .	192	208	7	393
6 . . . . .	159	241	240	160
7 . . . . .	7	393	77	323

Table VI shows that for seven treatments both the arrangements based on diagonal squares are likely to give greater residual variance than that for random arrangements. In the case of four, five and six treatments the findings are not consistent. For four treatments random arrangements are likely to give greater accuracy than that shown in chart 2 (1), while the one indicated in chart 2 (2) is likely to give more reliable information than random ones. In the case of six treatments this position is reversed. From this, it is clear that we are not sure of the exact type of balance to be effected to get greater accuracy. However, taking the eight diagonal squares, the residual error for six of the arrangements is likely to be greater than that for random arrangements.

Table VII gives the numbers of random arrangements having greater and less residual variance than that for Knut Vik squares.

TABLE VII  
*Comparison of random arrangements and Knut Vik squares*

No. of treatments	No. of random arrangements with residual variance			
	Greater than chart 7 (1)	Less than chart 7 (1)	Greater than chart 7 (2)	Less than chart 7 (2)
5 . . . . .	226	174	103	297
7 . . . . .	375	25	73	328

Here again, the results are not consistent. Of the two arrangements shown in chart 7 (1) and 7 (2), the latter is likely to give greater accuracy than the random designs, while in the case of the former the position is just the reverse. We are thus faced with the same difficulty as in the case of diagonal squares.

Table VIII shows the numbers of random arrangements with greater as well as with less residual variance than that for arrangements given in chart 10 for six treatments.

TABLE VIII

*Comparison between random arrangements and balanced Latin squares for six treatments with eight replications*

Arrangement referring chart 10	No. of random arrangements with variance	
	Greater	Less
1 . . . . .	176	224
2 . . . . .	147	253
3 . . . . .	310	90
4 . . . . .	281	119

Table VIII again shows that the claim of greater accuracy for balanced arrangements does not hold good for all balanced arrangements. It is possible to have several balanced arrangements and out of them some are more accurate while others are less accurate. In the present instance two cases are in favour and two are against balanced arrangements.

For four treatments in chart 3, balancing has been done in two directions, i.e. along rows and columns. Comparing this arrangement with the 300 random ones, it was found that 199 of them gave greater residual variance and the remaining 101 less. This finding is against the advantages claimed for balanced experiments.

Table IX gives the average treatment error and its variance for the 25 balanced arrangements and the random arrangements considered for the different cases.

Table IX shows that, excepting the case of six treatments, the average treatment errors are more for balanced arrangements. It may be mentioned that the differences are not significant in any of the cases. The variances for the three cases are almost the same. The treatment error and its variance are less for six treatments only. This leads to the conclusion that, on the whole, balanced arrangements are likely to give less accurate results. It may, however, be mentioned that the evidence available is not sufficient to say definitely one way or the other.

TABLE IX

*Average treatment error and its variance for random and balanced arrangements*

No. of treatments	Average variance		Variance of variance	
	Balanced	Random	Balanced	Random
4 . . . . .	387	314	44,553	53,452
5 . . . . .	188	151	9,442	5,466
6 . . . . .	84	102	1,538	3,819
7 . . . . .	114	104	2,308	3,246

The discussions in the preceding paragraphs show that it is difficult to say in advance which type of arrangement is likely to give more reliable information. During the course of the present investigation we have come across with a number of balanced designs which are likely to give more accurate results than random arrangements. But when laying out an experiment, it is not possible to get at this particular design. Even if the balancing principle is adopted, as has already been pointed out, there are different ways of effecting this balance. In laying out an experiment one of the balanced arrangements will have to be selected at random, as in the case of randomized blocks. The present investigation has not given us conclusive evidence to prefer one design to the other. Under these circumstances the best thing appears to be to have a design in which both the principles are combined, and we get it in Latin squares.

## SUMMARY

It has been claimed by Gosset that balanced arrangements are likely to be more accurate than random arrangements. The wheat uniformity trial discussed in a previous paper has been used to investigate the relative merits of random and balanced arrangements. The investigation covers the cases of four, five, six and seven treatments with six, six, eight and six replications, respectively, from different sections of the uniformity trial. It has been found that it is very difficult to say which of them is better. However, there is some tendency for the randomized arrangements to give more accurate results.

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### III. DISTRIBUTIONS OF VARIANCES AND RATIO OF VARIANCES

BY

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IT is well known that the ratio of variances test generally used for examining the significance of biological and agricultural data is based on the 'normal' theory. In actual practice the distribution of the parent population may either be not 'normal' or not known. In the case of field experiments we are not in a position to have any correct idea of the distribution of the population. In such cases it is essential to know whether the test usually applied is valid or not. This can be seen by examining the distribution of the ratio of variances (i) between and within groups of samples drawn from the same population, the distribution of which is unknown and (ii) between dummy treatments and residual error for the case of a uniformity trial data, distributing the treatments at random. As regards (i) it has already been shown by Pearson [1931] both from theoretical and experimental points of view that the distribution of the ratio of variances between and within groups of samples from the same population is not very sensitive to changes in the population form and that the 'normal' theory tests can be used with greater confidence than others, when dealing with populations whose distribution laws are unknown. Regarding (ii) Eden and Yates carried out some investigations on the validity of Fisher's  $z$ -test when applied to an actual example of non-normal data. The data consisted of height measurements of barley selected at random from various nitrogenous fertilizers, and they found the observed distribution agreeing satisfactorily with the theoretical distribution. But they have not clearly mentioned how the 24 permutations of the treatments A, B, C and D have been arranged and in the absence of this information it is not known whether the samples are biased or not.

In the present paper an attempt has been made to get some information regarding the validity of the ratio of variances test as applied to randomized blocks on the basis of the wheat uniformity trial, i.e. on item (ii) mentioned above. Incidentally the distributions of the variances for the dummy treatments and the residual error have also been compared with those of the 'normal' theory.

#### MATERIAL

Random arrangements for the different cases mentioned in part II have been taken advantage of to examine the agreement or the divergence between the theoretical distributions of the variances and the ratio of variances as compared to what is actually observed under field conditions.

#### METHODS AND DISCUSSIONS

*Variances.*—The frequency distributions of the ratio of the variances of the different samples to their mean value have been given in Tables I—IV for the treatment and the residual errors for the four cases discussed in part II. The expected values are calculated by using the distribution law

of variances on the basis of the 'normal' theory. It will be seen that this distribution will involve the variance of the population which is unknown. This has been assumed to be equal to the mean variance for the different samples. The error involved in such an assumption can be shown to be very small.

TABLE I  
Frequency distribution  
(4 treatments  $\times$  6 replications)

For treatment error				For residual error			
Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$	Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$
0-25	6	8.7	0.838	0-170	3	23.7	35.273
50	14	14.1	0.001	180	2	6.3	
75	16	16.5	0.015	190	1	6.9	
100	16	17.1	0.071	200	2	7.8	
125	16	17.4	0.113	210	2	8.7	
150	12	16.8	1.371	220	2	9.0	5.44
175	20	16.5	0.742	230	3	9.6	4.54
200	12	15.6	0.831	240	8	10.2	0.47
225	19	14.7	1.258	250	5	10.8	3.11
250	13	13.8	0.046	260	7	10.8	1.34
275	7	12.9	2.698	270	7	10.8	1.34
300	15	12.0	0.750	280	8	11.1	0.87
325	7	11.1	1.514	290	18	10.8	4.80
350	20	10.2	9.416	300	22	10.8	11.61
375	11	9.6	0.204	310	26	10.5	22.88
400	8	8.7	0.056	320	24	10.2	18.67
425	11	7.8	1.313	330	18	9.9	6.63
450	12	7.2	3.200	340	33	9.6	57.04
475	6	6.6	0.055	350	32	9.3	55.41
500	9	6.0	1.500	360	32	8.7	62.40
525	4	5.4	0.363	370	30	8.1	59.21
550	6	5.1	0.159	380	15	86.4	59.00
575	3	4.5	0.500				
600	3	4.2	0.343				
625	3	3.6	0.100				
650	6	3.3	2.209				
675	2	3.0	0.333				
700	1	3.0	1.333				
725	3	2.4	0.150				
750	5	2.1	4.005				
775	0	2.1	2.100				
800	2	1.8	0.022				
900	4	5.7	1.089				
1000	4	3.6					
Upwards	4	6.9					
Total	300	300	38.698	Total	300	300	410.03

TABLE II

*Frequency distribution*

(5 treatments × 6 replications)

For treatment error				For residual error			
Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$	Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$
0—20	9	12.0	0.750	0—96	4 }	47.6 }	45.306
40	24	28.0	0.571	99	3 }	6.8 }	
60	21	36.0	6.250	102	2 }	7.6 }	
80	41	38.4	0.176	105	3	8.0	3.125
100	42	38.4	0.338	108	3	8.4	3.471
120	44	35.6	1.982	111	3	9.2	4.178
140	36	32.8	0.312	114	4	9.2	2.939
160	24	28.8	0.800	117	3	9.6	4.538
180	32	25.2	1.835	120	10	10.0	0
200	22	21.6	0.007	123	8	10.1	0.437
220	27	18.0	4.500	126	5	10.4	2.804
240	9	15.6	2.792	129	11	10.5	0.024
260	19	12.8	3.003	132	12	10.6	0.185
280	6	10.8	2.133	135	8	10.6	0.638
300	13	8.8	2.005	138	18	10.6	5.166
320	7	6.8	0.006	141	26	10.5	22.881
340	5	6.0	0.167	144	8	10.4	0.554
360	5	4.8	0.008	147	29	10.3	33.950
380	4	4.0	0	150	16	10.1	3.447
400	3 }	3.2 }	2.010	153	26	9.9	26.183
420	3 }	2.4 }		156	22	9.6	16.017
440	2 }	2.0 }		159	39	9.2	96.526
500	2 }	8.0 }		162	29	9.2	42.613
				165	34	8.8	72.164
				168	28	8.4	45.733
				171	13	8.4	2.519
				174	20	7.6	20.232
				177	12 }	7.2 }	83.959
				180	1 }	101.2 }	
Total .	400	400	29.645	Total .	400	400	539.589

TABLE III  
*Frequency distribution*  
 (6 treatments  $\times$  8 replications)

For treatment error				For residual error			
Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$	Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$
0—20	9	14.4	2.025	0—80	5	66.7	68.347
25	6	8.8	0.891	82	3	9.8	
30	8	10.4	0.554	84	2	10.7	
35	8	11.6	1.117	86	4	11.2	
40	20	13.2	3.503	88	5	11.7	4.663
45	19	13.7	2.050	90	10	12.2	3.869
50	19	14.5	1.397	92	11	12.5	0.390
55	12	14.8	0.530	94	9	12.9	0.189
60	11	15.0	1.067	96	23	13.1	1.174
65	18	15.1	0.557	98	19	13.1	7.586
70	15	15.0	0	100	22	13.1	2.646
75	16	14.7	0.115	102	24	13.1	5.992
80	13	14.4	0.136	104	32	12.9	9.140
85	14	14.0	0	106	36	12.9	28.435
90	14	13.6	0.012	108	38	12.6	43.314
95	12	12.8	0.050	110	45	12.3	53.698
100	12	12.8	0.050	112	40	12.0	91.274
105	13	11.6	0.169	114	47	11.6	70.078
110	13	11.6	0.169	116	16	11.0	117.302
115	12	10.4	0.246	118	9	10.5	2.841
120	7	10.0	0.900			117.0	99.682
125	9	10.0	0.100				
130	7	8.8	0.368				
135	11	8.4	0.805				
140	4	7.6	1.705				
145	8	7.6	0.021				
150	7	6.8	0.006				
155	6	6.4	0.025				
160	10	5.6	3.457				
165	7	5.6	0.350				
170	8	5.2	1.508				
175	6	4.8	0.300				
180	3	4.4	0.445				
185	3	4.0	0.250				
190	3	3.6	0.100				
195	5	3.2	1.013				
200	3	3.2	0.013				
205	4	2.8	0.514				
210	5	2.8	1.729				
215	1	2.4	0.817				
Upwards .	19	24.4	1.195				
Total .	400	400	30.259	Total .	400	400	610.630

**TABLE IV**  
*Frequency distribution*  
 (7 treatments  $\times$  6 replications)

For treatment error				For residual error			
Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$	Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$
0—10	2 }	1.6 }	0.233	0—75	5 }	48.0 }	45.563
20	5 }	6.8 }		77	2 }	7.6 }	
30	17	14.4	0.469	79	3 }	8.4 }	
40	26	21.2	1.087	81	4	8.8	2.618
50	13	26.4	6.802	83	5	9.6	2.204
60	29	29.2	0.001	85	4	10.0	3.600
70	32	31.2	0.021	87	1	10.5	8.595
80	26	30.8	0.748	89	3	11.0	5.818
90	25	29.6	0.715	91	10	11.3	0.150
100	35	28.0	1.750	93	7	11.5	1.761
110	21	25.6	0.827	95	15	11.8	0.868
120	32	23.2	3.338	97	17	11.9	2.186
130	28	20.4	2.831	99	19	12.0	4.083
140	14	18.0	0.889	101	15	12.0	0.750
150	20	15.2	1.516	103	28	11.9	21.782
160	19	13.2	2.548	105	30	11.7	28.623
170	12	11.2	0.057	107	24	11.6	13.255
180	8	9.6	0.267	109	32	11.2	38.629
190	9	8.0	0.125	111	29	11.2	28.289
200	3	6.8	2.124	113	24	10.8	16.133
210	1	5.6	3.779	115	32	10.4	44.861
220	4	4.4	0.036	117	29	9.6	39.204
230	5	3.6	0.544	119	16	9.6	4.267
240	4	3.2	0.200	121	24	9.2	23.809
250	3 }	2.4 }	0.613	123	15	8.8	4.368
260	2 }	2.0 }		125	6	8.0 }	86.092
270	1 }	1.6 }		127	1	91.6 }	
Upwards .	4 }	6.8 }					
Total .	400	400	31.520	Total .	400	400	427.508

The values of  $\chi^2$  between the observed and the theoretical frequency distribution for the residual and the dummy treatment variances are given in Table V.

Table V shows that the observed distribution of the residual variance is not in accordance with the theoretical distribution. It is interesting to note that the observed distribution of the variance for dummy treatments is not significantly different from that of the theoretical distribution. This might probably be due to the fact that we are dealing with the distribution of variances based on the means.

TABLE V  
 $\chi^2$  for the treatment and the residual variances

No. of treatments	Residual variance		Variance for dummy treatments	
	Observed	Theoretical at 5 per cent level	Observed	Theoretical at 5 per cent level
7 . . . . .	427.5	40.1	31.5	41.3
6 . . . . .	610.6	31.4	30.3	> 43.8
5 . . . . .	539.6	42.6	29.6	35.2
4 . . . . .	410.0	33.9	38.7	> 43.8

*Ratio of variances.*—We have now seen that of the two variances one follows the 'normal' theory, while the other does not. It is now worth while to examine the effect of this deviation on the ratio of variances test. Table VI gives the observed and the theoretical frequencies of the ratio for the four hypothetical experiments discussed before.  $\chi^2$  and  $P(\chi^2)$  have also been given at the end of the table.

From Table VI it is clear that there is no reason to believe that the ratio of variances test is inapplicable in the case of data the distribution of which is unknown.

This conclusion is in full agreement with those of Pearson, and Eden and Yates. Pearson [1931] after extensive investigations on some non-normal data came to the conclusion that the analysis of variance is applicable over a fairly wide range of non-normality, provided the degree of freedom for the residual error is not small. Eden and Yates [1933] also found the distribution of  $z$  for 1000 random samples agreeing satisfactorily with the theoretical distribution.

### SUMMARY

The distributions of variances for (i) the treatments, (ii) the residual error and (iii) their ratio, have been investigated for experiments involving four, five, six and seven treatments, distributing dummy treatments at random on data computed from a uniformity trial. (The treatment variances are distributed in accordance with the 'normal' theory, while it is not so in the case of the residual error. As regards the ratio, the observed distributions are fairly in agreement with the theoretical distributions on the basis of the 'normal' law.

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TABLE VI  
*Frequency distribution for the ratio of variances*

4 treatments with 6 replications				5 treatments with 6 replications				6 treatments with 8 replications				7 treatments with 6 replications			
Classes	Frequency		$\frac{(O-C)^2}{C}$	Classes	Frequency		$\frac{(O-C)^2}{C}$	Classes	Frequency		$\frac{(O-C)^2}{C}$	Frequency		$\frac{(O-C)^2}{C}$	
	Observed	Calculated			Observed	Calculated			Observed	Calculated		Observed	Calculated		
0—15	23	21.6	0.1	0—125	11	11.6	0.0	0—125	6	6.2	0.0	6	3.4	2.0	
15—30	32	38.5	1.1	.250	26	26.0	0.0	.250	15	19.6	1.1	21	14.6	3.8	
30—45	28	23.6	0.8	.375	22	32.9	3.6	.375	38	29.4	2.5	29	26.4	0.3	
45—60	32	28.8	0.4	.500	38	35.1	0.2	.500	42	35.4	1.2	28	34.3	1.2	
60—75	26	26.0	0.0	.625	40	34.4	0.9	.625	42	36.8	0.7	42	37.8	0.5	
75—90	17	22.2	1.2	.750	41	32.2	2.4	.750	33	35.9	0.2	29	37.8	2.0	
90—105	16	19.4	0.6	.875	27	29.4	0.2	.875	32	33.5	0.1	40	35.7	0.5	
105—120	24	16.7	3.2	1.000	23	26.2	0.4	1.000	30	30.1	0.0	28	32.5	0.6	
120—135	14	14.4	0.0	1.125	24	23.1	0.0	1.125	26	26.8	0.0	29	28.7	0.0	
135—150	16	12.3	1.1	1.250	21	20.4	0.0	1.250	18	23.2	2.2	30	25.0	1.0	
150—165	12	10.4	0.2	1.375	8	17.5	5.2	1.375	19	19.8	0.0	20	22.8	0.2	
165—180	12	8.9	1.1	1.500	25	15.3	6.2	1.500	13	17.0	0.9	11	16.7	1.9	
180—195	4	7.7	1.8	1.625	16	13.1	0.6	1.625	18	14.4	0.1	23	14.8	4.5	
195—210	5	6.5	0.3	1.750	7	11.4	1.7	1.750	16	12.1	1.3	15	12.5	0.5	
210—225	3	5.6	1.2	1.875	5	9.3	2.4	1.875	13	10.0	0.9	7	10.2	1.0	
225—240	2	4.8	1.6	2.000	13	8.3	2.7	2.000	6	8.4	0.7	7	8.4	0.2	
240—255	6	4.1	0.9	2.125	6	7.3	2.2	2.125	5	7.0	0.6	8	7.0	0.1	
255—270	3	3.6	0.1	2.250	3	6.2	1.7	2.250	8	5.8	0.8	1	5.7	3.9	
270—285	2	3.1	0.4	2.375	3	5.3	1.0	2.375	7	4.8	1.0	2	4.7	1.6	
285—300	2	2.6	0.1	2.500	9	4.6	4.2	2.500	3	4.0	0.3	1	3.8	2.1	
300—315	2	2.3	0.0	2.625	6	3.9	1.1	2.625	3	3.3	0.0	3	3.1	0.0	
315—330	5	2.0	4.5	2.750	1	3.4	1.7	2.750	2	2.8	0.2	3	2.6	0.1	
330—345	0	1.7	1.7	2.875	3	2.9	0.0	2.875	2	2.3	0.0	5	2.1	4.0	
345—360	2	1.5	0.1	3.000	2	2.5	0.1	3.000	2	1.9	0.0	1	1.7	0.3	
360—375	2	2.5	0.1	3.125	3	2.2	0.3	3.125	3	2.2	0.0	4	1.4	4.8	
375—390	4	4.0	0.0	3.250	2	1.9	0.0	3.250	2	1.9	0.0	0	0	0.0	
390—405	3	2.9	0.0	3.375	1	1.6	0.2	3.375	3	9.5	0.2	2	6.8	0.0	
405—420	3	2.3	0.2	3.500	2	1.4	0.3	3.500	5	5	0.3	0	0	0.0	
420—435				3.625 and above	4	3.2	0.2	3.625 and above							
435—450					8	6.9	0.2								
450—465															
465—480															
480—495															
495—510															
510—525															
525—540															
540—555															
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1125—1140															
1140—1155															
1155—1170															
1170—1185															
1185—1200															
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1320—1335															
1335—1350															
1350—1365															
1365—1380															
1380—1395															
1395—1410															
1410—1425															
1425—1440															
1440—1455															
1455—1470															
1470—1485															
1485—1500															
Total	300	300	$\chi^2 = 22.8$ $P = 0.74$	300	300	300	$\chi^2 = 37.7$ $P = 0.16$	400	400	400	$\chi^2 = 15.0$ $P = 0.94$	400	400	400	$\chi^2 = 36.1$ $P = 0.09$

# RESEARCH NOTE

## A MOSAIC DISEASE OF COWPEA

BY

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(With Plates VII and VIII and two text-figures)

**D**URING the study of the effect of mixed cropping on the incidence of root-rot disease of cotton at Lyallpur an experiment was laid out in which Punjab cowpea type 1 (*Vigna Catjang*) had been inter-cropped in between the rows of *desi* cotton variety Mollisoni 39. In addition, a border of cowpea 2 ft. in width was sown all round the mixed plots. Both cotton and cowpea were sown on 20 May 1940. The affected cowpea plants exhibited three groups of symptom pictures. The first symptoms of the disease described in this note appeared about five to six weeks after sowing. The cowpea plants at this stage had almost covered the entire cotton crop as well as the surface of the soil in mixed plots.

**GROUP I:** The most obvious symptom is the general stunting of the affected cowpea plant. The symptoms are more marked in the upper portion of the plant. A prominent feature is the appearance of thick wrinkled leaves. The veins of the affected leaves are usually translucent when seen against light. After sometime mottling of leaves appears. This consists of small irregular light green areas which in parts are almost devoid of chlorophyll. Later on these turn yellow and the leaf-surface at this stage is a combination of yellow and green patches. The dark green areas are sometimes raised and look like blisters. The yellow patches are more marked on the upper surface of the affected leaf. Mottling and deformity of the leaf is usually accompanied by waving of the margins.

**GROUP II:** In some plants universal yellowing of the leaves is more prominent and the leaves are neither abnormally thick nor highly distorted.

**GROUP III:** There are other plants, the leaves of which show yellow and green patches. The mottling is very conspicuous. The diseased leaves frequently develop pale to brilliant yellow areas which later turn brownish in parts. The veins, including the mid-rib, turn reddish and look like dark red streaks. The most characteristic feature is the presence of dark brown to reddish spots about 1—2 mm. across on the upper surface of the affected leaves.

The common feature is the general stunting of the diseased plants and the affected leaves in due course drop off. Plate VII, figs. 1 and 2 and Plate VIII show symptoms exhibited by three groups of plants.

It may be mentioned that the naturally infected plants were seen both in the Botanical Experimental area and the Students Farm at Lyallpur where cowpea (Punjab type 3) alone had been sown. In the mixed cropping experiments the cotton plants appeared to be quite healthy and normal. About 15 per cent of the cowpea plants were affected.

### *Histology*

Transverse sections of the young wrinkled diseased leaves taken from the upper portion of the plant showing stunting and symptom picture of the first group and also leaves of a healthy plant were cut and examined microscopically. The affected leaf is much thicker than the normal leaf. The margin of the affected leaf is irregular and wavy and the cuticle is fused with the epidermal layer at various places; the epidermal cells are not defined but the cuticle and the epidermis is regular and marked in the healthy leaf which has a regular outline. In the affected leaf the normal palisade cells are small in number and the palisade tissue is neither continuous nor regular, whereas in the case of unaffected leaf the palisade tissue is regular. The palisade cells of the affected leaf have very few chloroplasts, whereas those of healthy leaves are full of them. The spongy parenchyma in the affected leaf is adversely affected. The sclerenchyma cells of the diseased leaf are thicker and larger in size than those of healthy leaves. The vascular bundles in the affected leaves are scattered and are not arranged regularly. The xylem vessels tend to be thicker and larger than in the healthy normal leaf. In the affected leaf a large number of elongated and irregular cells develop which appear to have partly taken the place of spongy and palisade tissue. Figs. 1 and 2 show transverse sections through a diseased and a healthy leaflet respectively.

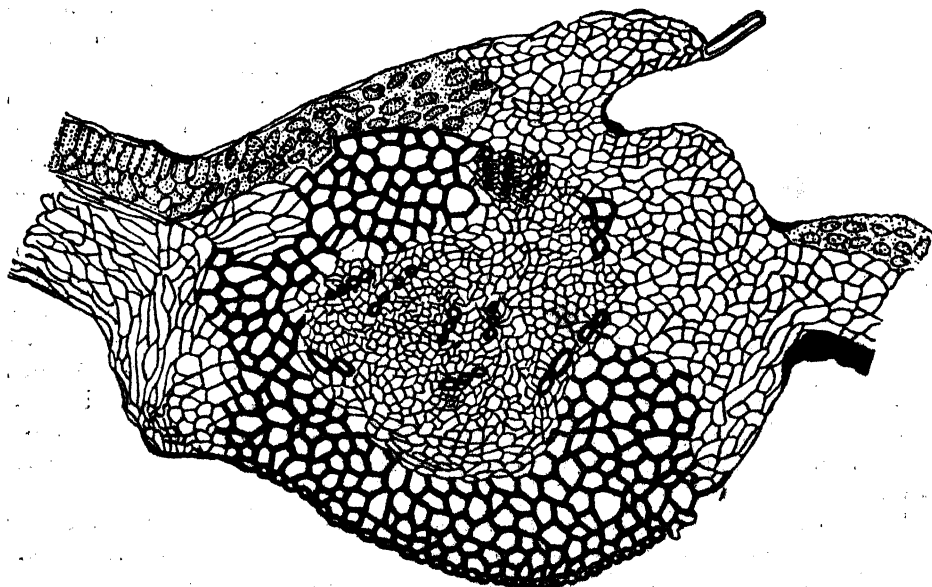
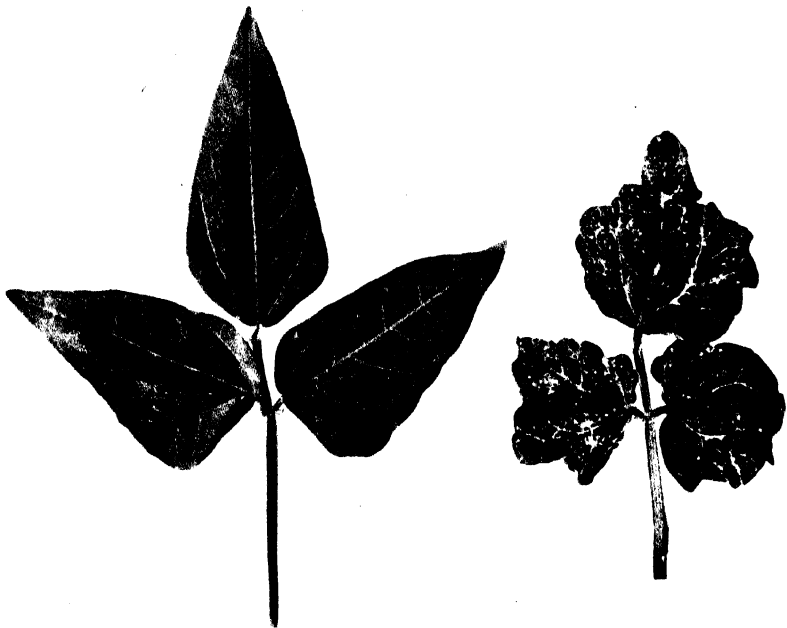


FIG. 1. Transverse section of a malformed cowpea leaflet ( $\times 50$ )



Healthy

Diseased

FIG. 1. Leaves of group I plants



Healthy

Diseased

FIG. 2. Leaves of group II plants



Diseased

Healthy

Leaves of group III plants

### *Infectivity*

Isolations made from the diseased material did not yield any organism. Microscopic examination also did not reveal the presence of fungal hyphae.

The juice of the plants was extracted in a sterilized pestle and mortar, strained through fine muslin cloth and centrifuged to remove the

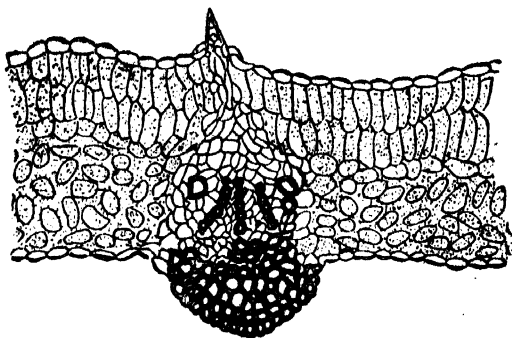


FIG. 2. Transverse section of a healthy leaflet ( $\times 50$ )

cell-debris. Inoculations were made by smearing the leaves with the juice by means of a spatula on which cloth had been wrapped and pricking the smeared leaves. Juice was also introduced by means of a syringe, but as the first method proved quite handy and successful it was continued. Young cowpea plants raised in sterilized soil inside large glass-walled chambers were inoculated with the extracts of the washed diseased plant leaves. Checks were inoculated with the juice of healthy plants.

The juice from leaves of plants showing all the three types of symptoms described above was used separately for inoculation purposes, but it was observed that in all cases yellow mottling appeared on the leaves of inoculated plants within five days, whereas the controls remained healthy. In these preliminary inoculation tests the distortion of the leaves was not observed to have been reproduced.

Holmes [1939] mentions a mosaic disease of cowpea induced by artificial inoculation with cucumber-mosaic virus, cowpea-mottling strain, but does not mention the occurrence of the disease in nature. It has not been determined whether the disease referred to in the note is the same. Smith [1924] has, however, mentioned the occurrence in Louisiana, Arkansas and Indiana of a cowpea mosaic causing mottling and crinkling of the leaves.

### REFERENCES

- Holmes, F. O. (1939). *Handbook of Phytopathogenic Viruses*, pp. 39-40: Burgess Pub. Co., Minneapolis  
Smith, C. E. (1924). *Science* 40, 268

## REVIEWS

**Annual Review of Biochemical and Allied Research in India, Vol. XI, 1940.**  
Society of Biological Chemists, India, Bangalore : Price Rs. 3 or 6d.

**T**HE review covers a wide field and consists of 16 sections the subject-matter of which has an important bearing on agricultural science, such as enzymes, vitamins, animal nutrition, adulteration of foods, phytopathology, soils, fertilizers and manures, biochemical and allied industries, etc. Only a brief mention of a few of the items is made in this short review.

The contributions made in the field of enzymes during the year relate to the nature of carboxylase, zymohexase, liver aldehyde oxidase, cytochrome oxidase and other oxidizing enzymes. The work of Indian workers from the Biochemical Laboratories, Cambridge, on biological oxidations, also deserves notice.

The work of the Bengal Nutrition Committee and the occurrence of a widespread famine in the south-eastern districts of the Punjab raise the question of Vitamin-A deficiency. In the famine areas scurvy and night-blindness have been widespread. While scurvy was largely controlled by the use of *amla* powder, no such cheap and potent source of vitamin-A was available for mass distribution. War stopped the import of cod-liver oil. Scientists therefore began to look to the life in the numerous rivers, bays, canals and tanks of India for any available supply of vitamin A.

In 1940, there was greater interest in putting down the adulteration of food and drugs. The enactment of the Drugs Act and the formation of the Central Committee for Food Standards were notable events. Equally encouraging was the output of scientific work in this field.

The year marks an increasing interest in researches on applied plant physiology, such as in vernalization, water relation, the influence of mineral elements, etc. Useful contributions were made on germination and viability of seeds, respiration in light, photo-periodism and radiation effects on the growth of plants.

In the field of entomology, several studies were made dealing with insect pests of cotton and sugarcane. Further, *Schistocerca gregaria* Forsk. has been found to be the locust *par excellence* of India not only by the frequency of its visitations, but also by the extent and severity of its attacks. The locust problem is now recognized to be an international one and in setting out India's part in investigations on this pest, considerable advance has been made in the study of the bionomics of the desert locust, the nature of its habitat and the conditions under which new outbreaks may occur.

The second world war has brought in its train great slackening of all scientific research except that connected with the war effort. This has been due to the difficulties in obtaining requisite supplies of suitable chemicals and apparatus. In the domain of the chemistry of plant products, the paucity of research by Indian chemists is particularly noticeable during the year.

However, important investigations have been carried out in this line on essential oils, fixed oils and waxes, lactones and glucosides comprising bitter principles of plants, plant-colouring matters and alkaloids.

Under biochemical and allied industries, interest continues to be centred in the utilization of molasses for the preparation of chemicals. The spade work in connection with the manufacture and use of power alcohol may now be considered to be complete with the enforcement of the U. P. Power Alcohol Act of 1940, making it compulsory for every petrol distributor within certain areas to add 20 volumes of power alcohol to every 80 volumes of petrol, before retailing the latter to the public. The industry has, however, received a temporary setback, as it is not possible to import the necessary plant.

The interest in the manifold problems presented by the soils has continued as is manifest in the impressive volume of work in the year.

A comparative study of the different soils of India and profiles for their classification into broad groups in relation to the world scheme of classification as also to the cultural and fertilizer practices was in progress. In order to gain a general idea of the evolutionary status of the Indian soils under varied geological and climatic influences, three soil maps have been prepared there, based on (1) agricultural and colour nomenclature, (2) the relative nitrifying power of surface soils, and (3) climatic differences.

Molasses, a waste product of the sugar industry, has found useful application as manure, and so also filter-press cake, and compost made up of press-mud, cane-trash and bagasse.

Fertilizer trials were continued during the year. In Bihar an application of 60 lb. of N and 60 lb. of  $P_2O_5$  to sugarcane gave a net profit of Rs. 35-40 per acre, in the Central Provinces the highest net profit per acre was with 20 lb. of  $P_2O_5$  and amounted to Rs. 3-12 only per acre; while in Orissa doses of nitrogen from 20 to 40 lb. gave increased yields which, however, did not pay for the cost of manure. The application of phosphatic manures to Assam soils gave profitable response in crop yields.

Dry cultivation offers the largest scope for increasing the wealth of the country, especially in Mysore where 80 per cent of land is under dry cultivation.

*An Agricultural Testament* by Sir Albert Howard published during the year is a useful contribution to the careful study of Indian agricultural problems. The whole thesis of the publication is to show that for true agricultural success organic manure is essential, since it produces humus, and humus is necessary for mycorrhizal symbiosis between the plant roots and the soil which extensive experience has apparently shown to be fundamental. Plants grown under proper agricultural conditions, with ample aeration in presence of humus, are shown to be disease-resistant, and animals including human beings fed on such vegetables are also resistant to disease (B. V. N.).

## NOTES

### BOMBAY AGRICULTURAL PESTS AND DISEASES ACT, 1941

**T**HE *Bombay Government Gazette* of the 12th September 1941 publishes an Act to provide for the prevention of the introduction, spread or reappearance of insect pests, plant diseases and noxious weeds injurious to crops, plants or trees in the province of Bombay, to be known as the 'Bombay Agricultural Pests and Diseases Act, 1941'.

Four distinct forms of action under the Act for control of pests, diseases or weeds are envisaged. The provincial Government may, by notification in the official Gazette :—

- (1) declare that such pest, disease or weed is an insect pest, plant disease or noxious weed
- (2) specify the local area within which and the period during which such declaration shall remain in force
- (3) prohibit or restrict the removal of any plant or tree from one place to another, and
- (4) direct the carrying out of such preventive or remedial measures, including the destruction of any pest, disease or noxious weed or any crops, plants or trees, as the provincial Government may deem necessary, in order to eradicate such pest, disease or weed or to prevent its introduction, spread or reappearance

The Government will appoint Inspectors to enter upon any land or premises within a notified area, to ascertain the presence of insect pests, plant diseases or noxious weeds and to see that the measures advocated have been carried out. If the measures have not been taken, he is empowered to issue an order giving a time limit for their completion, against which order an appeal may be preferred with the Collector. In the event of continued failure, the Inspector himself may carry out the work, the cost being recovered as an arrear of land revenue. Compensation for trees or plants destroyed under a general or particular order will be granted. The amount of compensation shall be as follows :—

- (1) If a tree is infected with an insect pest or a plant disease, a sum not exceeding half the value of the tree.
- (2) If plants are grown so close together that they cannot be treated individually and healthy plants have also to be destroyed, a sum not exceeding three-fifths of the value.
- (3) If plants or trees are destroyed which though not infected at the time with an insect pest or a plant disease are, in the opinion of the Inspector, liable to such infection, a sum equal to the full value.

Cotton plants are excluded from compensation, as also plants and trees which in the opinion of the Inspector contracted infection due to negligence of the occupier in carrying out preventive or remedial measures mentioned in a notification.

Persons removing plants or trees in contravention of a notification, or failing to comply with a notice, or in any other way committing a breach of the provisions of the Act are liable to punishment with a fine which may extend up to Rs. 25.

The Act itself does not include the mention of any particular insect pest, disease, or weed, but a statement appended to the notification mentions a number of pests and diseases requiring attention, namely mildew, aphis, stem borer, cotton bollworm, grasshopper, coconut-tree pests, and *koleroga* disease of betel-nut palms. It is mentioned that owing to the failure of a few land owners or occupiers to cooperate, it has been impossible to eradicate these pests and diseases effectively, and it is in order to compel simultaneous action that the Act has been passed.

This Act appears to be modelled on the 'Madras Agricultural Pests and Diseases Act, 1919', which has proved of considerable help to the Madras Department of Agriculture in enforcing pest control measures in that province.

### MAYNARD-GANGA RAM PRIZE

APPLICATIONS are invited for the award of the Maynard-Ganga Ram Prize of Rs. 3,000 for a discovery or an invention or a new practical method which will tend to increase agricultural production in the Punjab on a paying basis. The prize is open to all, irrespective of caste, creed or nationality, and Government servants are also eligible for it. Essays and theses are not accepted. The prize will be awarded for something practically achieved as a result of work done after the prize was founded in 1925. In their applications competitors must give a clear account of the history of their invention or discovery and must produce clear evidence that it is the result of their own work. In the case of an improved crop details of parentage, evolution and history and a botanical description are necessary.

The Managing Committee reserves to itself the right of withholding or postponing the prize if no satisfactory achievement is reported to it, or to reduce the amount of the prize or to divide it if the quality of the entries justify this action.

Entries should reach the Director of Agriculture, Punjab, Lahore, not later than 31 December 1942.

THE Imperial Agricultural Bureaux have just issued the 10-year Subject and Author Index to *Horticultural Abstracts* 1931-40. Price about 25s. (No free issue.)

All orders should be sent direct to The Imperial Agricultural Bureaux, Central Sales Branch, Agricultural Research Building, Penglais, Aberystwyth, Wales.



# PLANT QUARANTINE NOTIFICATIONS

INDIA

*Form of special permit authorizing importation of insects*

[Prescribed by the Central Government under para. 2(a) of the Notification\*  
No. F.-193/40-A, dated 3 February 1941]

1. Name, designation and full address of the importer . . . . .
  2. Name of the insect species to be imported . . . . .
  3. Stage or states of the insect to be imported . . . . .
  4. Country from which importation is sought . . . . .
  5. Whether importation is intended by sea, land or air . . . . .
  6. Whether in its original home it is a weed pest, a parasite or a predator. . . . .
    - (i) Name (names) of the weed (weeds) on which it is a pest in the country of origin . . . . .
    - (ii) Name (names) of the pest (pests) on which it is a parasite or predator in the country of origin . . . . .
  7. Name, designation and address of the exporter . . . . .
  8. Quantity indented for . . . . .
  9. Purpose of importation . . . . .
- I authorize the importation. This permit will be valid up to.....

(Signature and designation of the  
certifying authority)

Date.....

[N.B.—It is expected that the permit will be obtained in advance of sending the order so that the imported material may not remain indefinitely in the warehouse for want of suitable permit.]

*Notification No. F. 193/40-A. (c), dated 12 August 1941 of the Government of India in the Department of Education, Health and Lands*

**I**N exercise of the powers conferred by sub-section (1) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government is pleased to direct that the following amendment shall be made in the Order

\*Published in this Journal, Vol. 11, Part II, page 322

published with the notification of the Government of India in the Department of Education, Health and Lands, No. F.-193/40-A., dated the 3rd February 1941, namely :—

In clause (b) of paragraph 3 of the said Order, after the word ' Orissa ' the words ' Jammu and Kashmir ' shall be inserted.

*Notification No. F. 15-11/41-A., dated 1 September 1941 of the Government of India in the Department of Education, Health and Lands*

**I**N exercise of the powers conferred by sub-section (1) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government is pleased to direct that the following further amendment shall be made in the Order published with the notification of the Government of India in the Department of Education, Health and Lands, No. F. 320-35-A., dated the 20th July 1936, namely :—

In sub-paragraph (2) of paragraph 9 of the said Order for the words and brackets ' (*Ceratostomela paradoxa* or *Thielaviopsis paradoxa*) ' the words and brackets ' *Ceratostomella paradoxa* (*Thielaviopsis paradoxa*) ' shall be substituted.

#### FOREIGN COUNTRIES

*Notice No. 2 of 1941 regarding plant quarantine regulations and import restrictions received in the Imperial Council of Agricultural Research*

**T**HE following plant quarantine regulations and import restrictions have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

##### 1. *Quarantine and other official announcements*

- (i) Service and Regulatory Announcements October-December 1940
- (ii) Fruit and Vegetable Quarantine of Puerto Rico
- (iii) Japanese Betel Quarantine

##### 2. *Summaries of plant quarantine import restrictions*

- (i) Plant Quarantine import restrictions of the Dominion of Canada
- (ii) Plant Quarantine and Import Restrictions of the Free City of Danzig previous measures abrogated
- (iii) Foot-and-mouth disease in Norway

##### 3. *Other announcements*

- (i) Government of Burma, Department of Agriculture and Forests Notification No. 141, dated the 2nd June 1941
- (ii) Government of Burma, Department of Agriculture and Forests Notification No. 182, dated the 25th June 1941 regarding import of live insects into Burma

## ERRATA

### THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE, VOL. IX, PART IV

Page 605—

Total solids in Table VIII signify residue left on drying the soil extract to constant weight and are not to be confused with the sum of the different water-soluble constituents each determined separately.

Table VIII, column 2, line 7, for '0·010' read '0·102'

Table VIII, column 9, line 5, for '0·095' read '0·038'

### THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE, VOL. XI, PART V

Pages 710-11 (Table III), columns 3, 4, 5, 6, 7 and 8 of the lower half of the table refer respectively to '*Hirsutum*', '*Herbaceum*', '*Arboreum*', '*Cernuum*', 'All cottons' and 'By analysis of variance'.



# ORIGINAL ARTICLES

## PROPERTIES OF SUB-FRACTIONS OF HYDROGEN CLAY PREPARED FROM INDIAN SOILS, I\*

BY

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(Received for publication on 25 August 1941)

(With six text-figures)

IN recent years, the properties of sub-fractions of clay having particles of different sizes have attracted considerable attention in consequence of the light they throw on soil formation processes [Brown and Byers, 1932; Bradfield, 1935; Bray, 1937]. Apart from this standpoint, variations in colloidal properties with the particle size, even when the mass chemical composition of the disperse phase, the clay, shows little or no change with the degree of dispersion, are of considerable theoretical interest. A study of the properties of sub-fractions of hydrogen clays separated from Indian soils has been undertaken by us with both these objects in view. The present paper is the first of a series which will deal with these studies.

### EXPERIMENTAL

(a) *Separation of sub-fractions from the entire clay fraction*—Particulars regarding the soils used are given below.

TABLE I  
*Particulars of soils used*

Laboratory No.	Description of soil	pH		Clay + loss on solution (per cent)	Silt (per cent)	Base-exchange capacity by Parker's [1929] method	Total exchangeable bases (S) by Rice Williams' [1932] method
		Aq. suspension	NKCl suspension				
22	Red lateritic soil from Government Agric. Farm, Dacca (Bengal) collected from a depth of 0 to 6 inches	5.12	4.21	22.8	30.1	8.4	4.8
34	Black soil from Government Experimental Farm, Akola, Berar (C. P.) collected from a depth of 0 to 9 inches	7.10	7.05	44.8	19.6	39.5	39.5

\*The results given in this paper have been taken from the published Annual Report for 1937-38 on the working of a scheme of research into the Properties of Colloid Soil Constituents financed by the Imperial Council of Agricultural Research, India

\*\*Senior Assistant Soil Chemist under the above scheme

From the entire clay fractions of the soils the following subfractions were separated by controlled centrifugal subsidence. A 'bucket type' centrifuge\* having a diameter of 25 cm. and capable of making 5,000 revolutions per minute was used.

TABLE II

*Equivalent spherical diameters of sub-fractions and their percentages in the entire clay fraction*

Soil	Reference number of sub-fraction	Percentage of sub-fraction in the entire clay	Limiting equivalent spherical diameters in microns	Reference number of corresponding hydrogen clay
1. Red lateritic soil . . . . .	1	25.10	0.26 and 2.0	L <sub>1</sub>
	2	9.60	0.16 and 0.26	L <sub>2</sub>
	3	65.40	< 0.16	L <sub>3</sub>
2. Black soil . . . . .	1	13.50	0.45 and 2.0	M <sub>1</sub>
	2	4.80	0.23 and 0.45	M <sub>2</sub>
	3	5.20	0.15 and 0.23	M <sub>3</sub>
	4	75.50	< 0.15	M <sub>4</sub>

The depth of sampling and the rate of revolution of the centrifuge were kept constant in separating the different fractions; only, the time of settling was varied. The limiting equivalent spherical diameters (given in column 4 of Table II) were calculated from Stokes' law under the following simplifying assumptions:

- (i) The different fractions have the same density.
- (ii) The particles settle under a uniform centrifugal force, viz. that obtaining at half height.
- (iii) The particles have a spherical symmetry.

The different subfractions were leached with dilute (0.05N) hydrochloric acid to obtain the corresponding hydrogen clays which were washed free from HCl and made up to suspensions containing 2.5 gm. of oven-dried (105°C.) material per litre. All measurements reported in this paper were made with these hydrogen clays.

(b) *Fusion analysis for Fe, Al and Si.*—The usual methods of soil and clay analysis were followed [Wright, 1937].

(c) *Electrometric titration of hydrogen clays.*—The technique of Mukherjee *et al.* [1936] was followed. In addition to hydrogen and quinhydrone electrodes used by them glass electrodes (Morton type) in conjunction with a Cambridge electrometer valve potentiometer reading directly to 2 millivolts were used.

\*A Sharples supercentrifuge is now being used for separating very fine sub-fractions

## RESULTS

(a) *Chemical composition*.—The results of fusion analysis are given in Table III.

TABLE III

*Chemical compositions of the entire hydrogen clay and its sub-fractions isolated from the Dacca soil*

Hydrogen clay	SiO <sub>2</sub> per cent	Al <sub>2</sub> O <sub>3</sub> per cent	Fe <sub>2</sub> O <sub>3</sub> per cent	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$
L *	51.2	36.0	11.9	2.40	1.99
L <sub>1</sub>	64.9	30.6	5.0	3.60	3.26
L <sub>2</sub>	49.6	36.5	12.6	2.31	1.85
L <sub>3</sub>	39.9	46.5	14.0	1.46	1.22

\*Obtained from the entire clay fraction

TABLE IV

*Chemical compositions of the entire hydrogen clay and its sub-fractions isolated from the Akola soil*

Hydrogen clay	SiO <sub>2</sub> per cent	Al <sub>2</sub> O <sub>3</sub> per cent	Fe <sub>2</sub> O <sub>3</sub> per cent	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$
M*	53.1	30.4	16.9	3.0	2.19
M <sub>1</sub>	65.8	24.2	9.8	4.6	3.7
M <sub>2</sub>	59.3	26.9	14.2	3.7	2.6
M <sub>3</sub>	52.1	30.6	18.0	2.9	2.1
M <sub>4</sub>	48.4	32.3	19.3	2.6	1.8

\*Obtained from the entire clay fraction

The percentage of silica decreases with diminishing particle size while that of alumina and ferric oxide increases. In consequence, the silica-alumina and silica-sesquioxide ratios decrease with diminishing particle size. The percentage of silica is definitely higher in the coarsest fraction of the red soil and the two coarse fractions of the black soil compared with the respective entire clays. The percentages of Al<sub>2</sub>O<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub> are, on the other hand, greater in the entire clays than in the above coarse fractions but lower than in the finer fractions.

The results of fusion analysis of the silt fractions given in Table V follow the regular sequence of variations in composition with the particle size within the clay fraction.

TABLE V  
*Chemical compositions of the silt of the Dacca and Akola soils*

	SiO <sub>2</sub> (per cent)	Al <sub>2</sub> O <sub>3</sub> (per cent)	Fe <sub>2</sub> O <sub>3</sub> (per cent)
Silt of the Dacca soil . . . .	90.4	6.76	1.42
Silt of the Akola soil . . . .	80.2	12.40	6.02

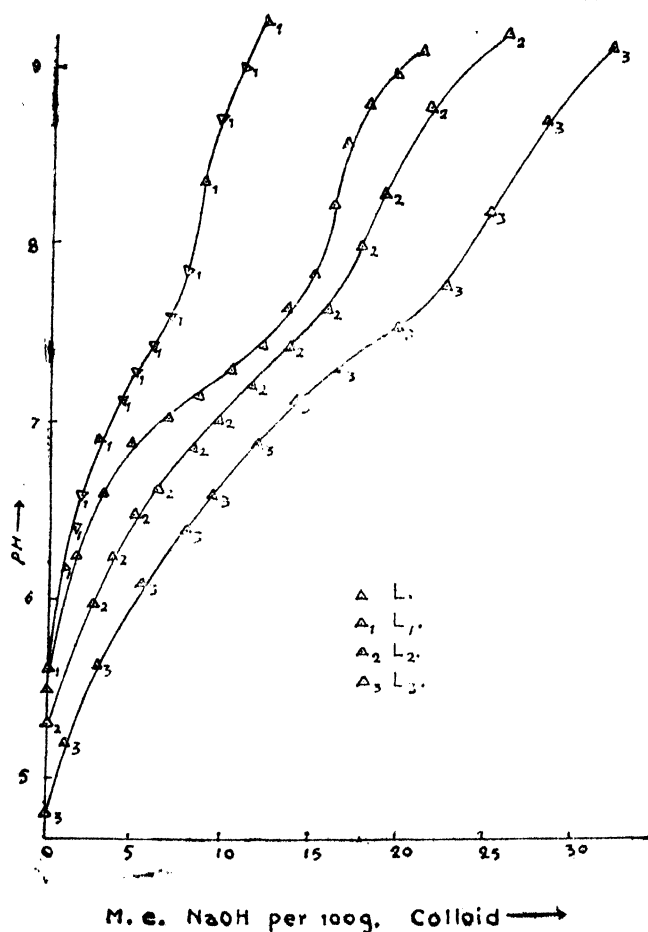


FIG. 1. Titration curves with NaOH of the entire hydrogen clay and its sub-fractions isolated from the Dacca lateritic soil

A similar increase in the percentage of silica in the silt fraction as compared with the 1-2  $\mu$  fraction was observed by Marshall [1935]. He attributed this to a greater percentage of free quartz in the silt.

The decrease in the percentage of silica with diminishing particle size indicates that the chemical weathering process attending the mechanical breakdown of the large particles is one of continued desilication which appears to be more pronounced with the red lateritic soil than the black soil in agreement with the more intense conditions of leaching under which the red soil has been formed compared with the other soil.

(b) *Free and total acids.*—Table VI gives the free acids calculated from the  $pH$ 's of 0.25 per cent suspensions and the total neutralizable acids calculated from the titration curves with different bases given in Figs. 1-6. The total acids have been calculated both at the inflexion points of the curves as also at  $pH$  7.0.

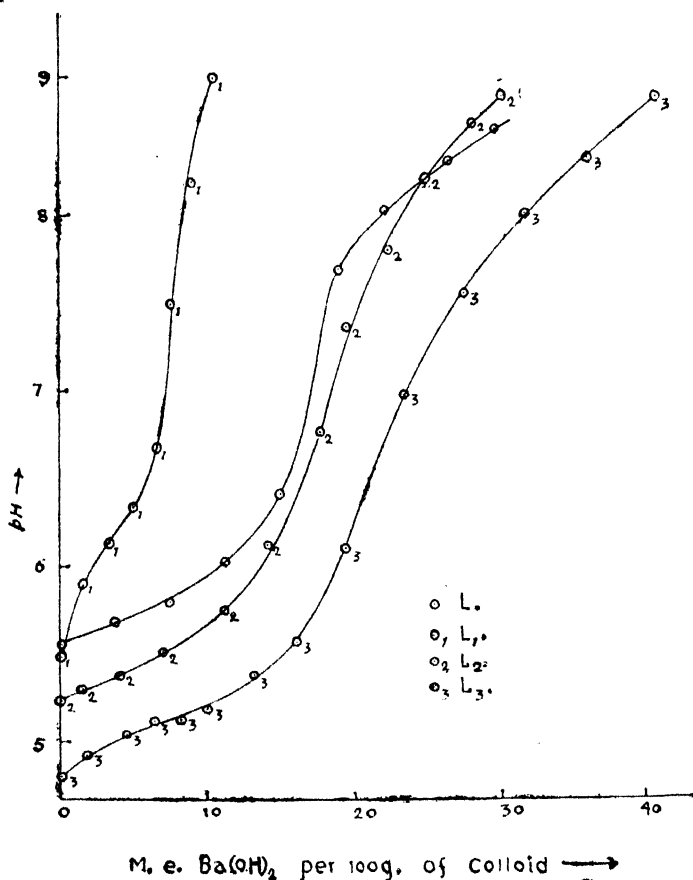


FIG. 2. Titration curves with  $Ba(OH)_2$  of the entire hydrogen clay and its sub-fractions isolated from the Dacca lateritic soil

Both the free and total acids increase with diminishing particle size.  $L_1$  and  $M_1$  obtained from the coarsest fractions have much smaller total acids than  $L$  and  $M$  obtained from the entire clays.  $L_2$  and  $L_3$  have greater total acids than  $L$ . Those of  $M_2$  and  $M_3$  are small compared with  $M$ .  $M_4$ , however, gives a definitely greater value than  $M$ . The larger total acid of  $M$  compared with  $M_1$ ,  $M_2$  and  $M_3$  is expected as the entire clay fraction is largely made up of the finest fraction (Table II) having the largest total acid.

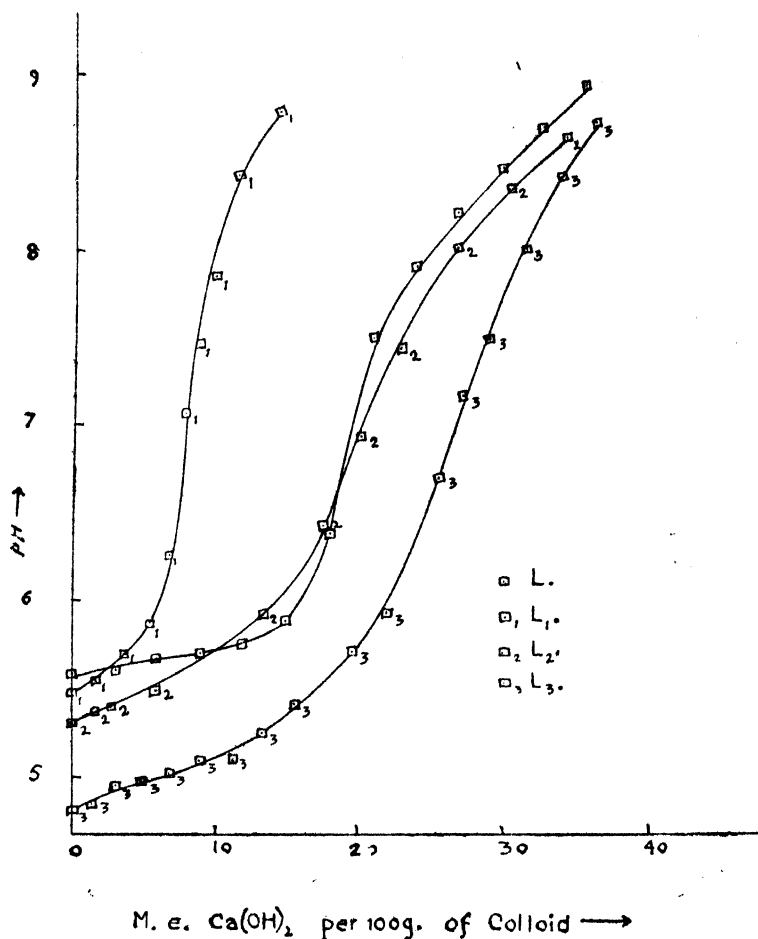


FIG. 3. Titration curves with  $\text{Ca(OH)}_2$  of the entire hydrogen clay and its sub-fractions isolated from the Dacca lateritic soil

TABLE VI

*Free and total acids of sub-fractions of hydrogen clay*

Hydrogen clay	$\frac{\text{SiO}_2 \text{ (molar)}}{\text{Al}_2\text{O}_3}$	Free acid in m. e. $\text{H}^+$ ions per 100 gm. colloid	Total acid in m. e. base per 100 gm. colloid using					
			NaOH		$\text{Ba(OH)}_2$		$\text{Ca(OH)}_2$	
			At inflexion point	At pH 7.0	At inflexion point	At pH 7.0	At inflexion point	At pH 7.0
$L_1$	3.60	0.07	8.33	3.30	7.0	7.0	8.0	8.0
$L_2$	2.31	0.17	17.60	9.03	18.0	18.6	20.2	20.2
$L_3$	1.46	0.60	24.00	12.65	19.6	23.8	26.0	26.6
$M_1$	4.60	0.08	3.80	3.70	2.8	5.0	3.5	5.3
$M_2$	3.70	0.21	14.00	10.00	12.6	13.0	13.6	14.7
$M_3$	2.9	1.10	36.5	31.50	30.5	33.5	31.5	38.5
$M_4$	2.5	4.38	86.0	86.00	86.0	97.0	90.0	98.5

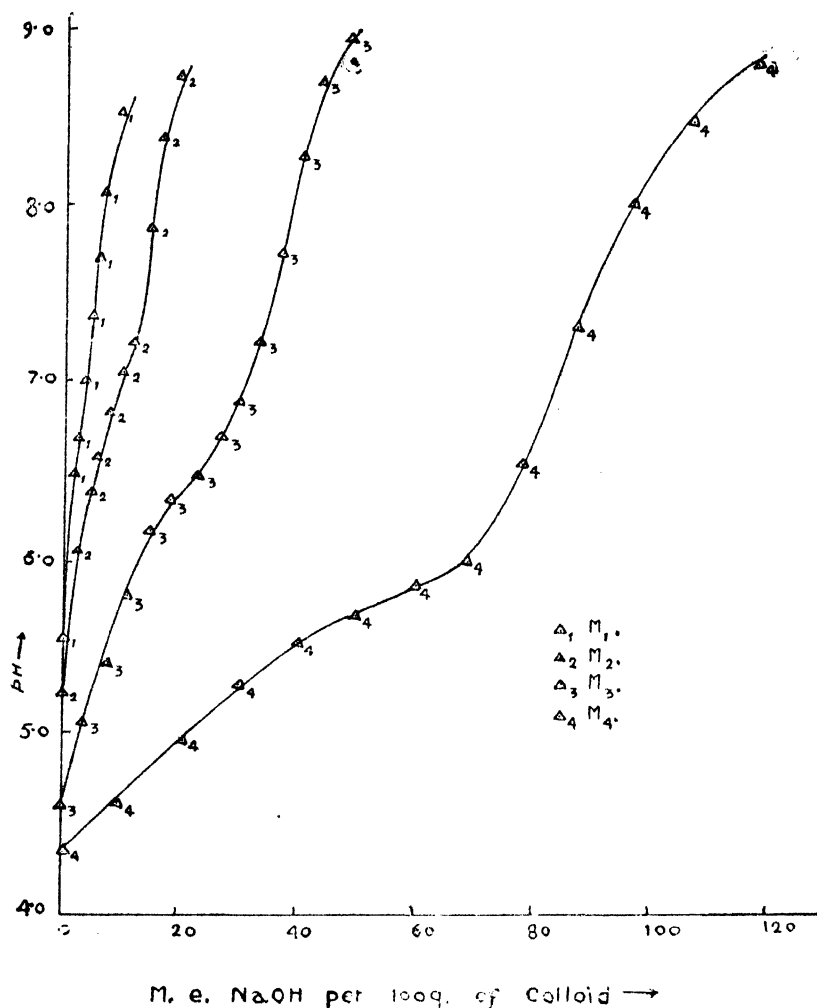


FIG. 4. Titration curves with NaOH of sub-fractions of hydrogen clay isolated from the Akola soil

In Table VII the observed total acids of L and M have been compared with their total acids calculated from those of the sub-fractions and the percentages of these sub-fractions in the entire clays.

TABLE VII

*Total acids of entire hydrogen clays calculated from their titration curves and from those of their sub-fractions*

Hydrogen clay	Total acid (at pH 7.0) in m. e. base per 100 gm. colloid using					
	NaOH		Ba(OH) <sub>2</sub>		Ca(OH) <sub>2</sub>	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
L	6.3	9.9	17.0	19.0	19.5	21.3
M	60.0	67.0	79.0	75.2	87.0	77.0

The observed and calculated values show a fair agreement ignoring the total acids of L with NaOH and of M with  $\text{Ca}(\text{OH})_2$ .

(c) *Form of titration curves.*—Previous work from this laboratory has dealt with the titration curves of hydrogen clays obtained from entire clay fractions [Mitra, 1936, 1940; Mukherjee, Mitra and Mukherjee, 1937]. A comparison of the titration curves of sub-fractions of hydrogen clay obtained from the same soil may afford useful information regarding the nature of the reactive acidic material present in the different fractions. Such comparative studies have not been previously made.

The NaOH curves of  $L_1$ ,  $L_2$  and  $L_3$  given in Fig. 1 are similar to one another and to that of L obtained from the entire clay fraction. This is also true of  $M_1$ ,  $M_2$ ,  $M_3$ ,  $M_4$  and M given in Fig. 4. This similarity suggests that the same acid is being titrated in the different sub-fractions. This conclusion, however, does not apparently harmonize (further discussed later) with their markedly different chemical compositions given in Tables III and IV.

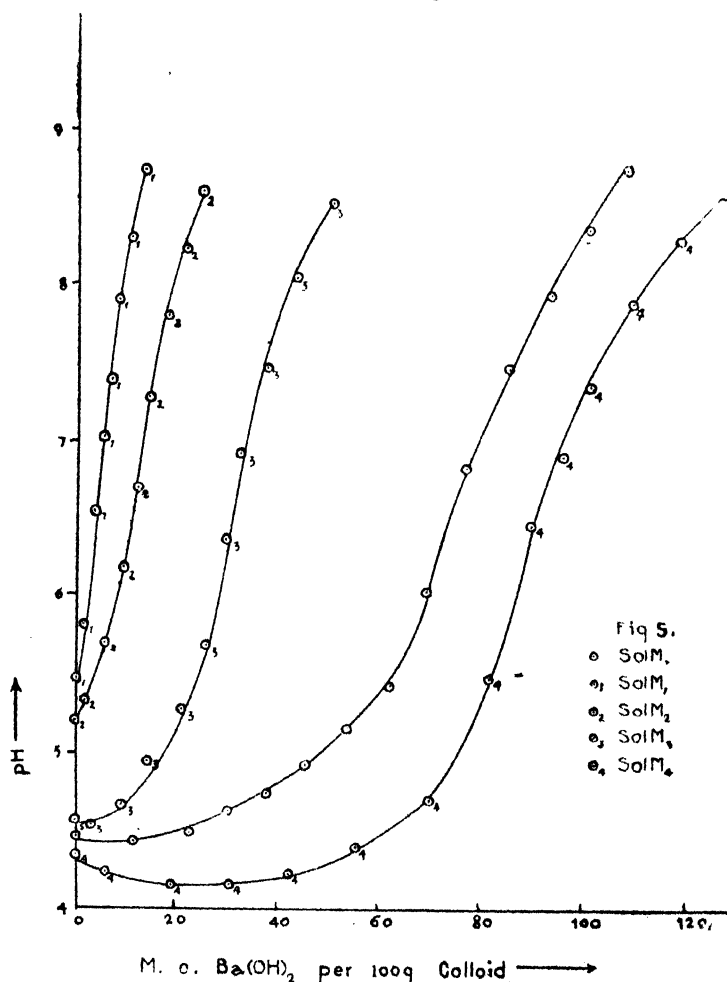


FIG. 5. Titration curves with  $\text{Ba}(\text{OH})_2$  of the entire hydrogen clay and its sub-fractions isolated from the Akola soil

The NaOH curves all have an apparent weak monobasic acid character. Table VIII shows the  $pH$  at their inflexion points and the apparent dissociation constants calculated from the  $pH$ 's at half neutralization.

TABLE VIII

*pH at inflexion and dissociation constants of hydrogen clays*

Sol	$pH$ at inflexion	$pH$ (= $pK$ ) at half neutralization	$K$
L	8.21	7.13	$7.41 \times 10^{-8}$
L <sub>1</sub>	8.10	7.15	$7.10 \times 10^{-8}$
L <sub>2</sub>	8.00	6.97	$1.07 \times 10^{-7}$
L <sub>3</sub>	8.03	6.96	$1.10 \times 10^{-7}$
M	7.49	6.20	$6.31 \times 10^{-7}$
M <sub>1</sub>	7.10	6.56	$2.75 \times 10^{-7}$
M <sub>2</sub>	7.63	6.66	$2.20 \times 10^{-7}$
M <sub>3</sub>	7.60	6.26	$5.50 \times 10^{-7}$
M <sub>4</sub>	7.00	5.56	$2.75 \times 10^{-6}$

The inflexion points in the titration curves of L, L<sub>1</sub>, L<sub>2</sub> and L<sub>3</sub> occur within a narrow range of  $pH$  (8.00–8.21) and the dissociation constants calculated from the curves are in fair agreement. With M, M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> the inflexion points are located within a wider range of  $pH$  (7.00–7.63) though the agreement between the dissociation constants is not unsatisfactory excepting the finest fraction (M<sub>4</sub>) whose dissociation constant is ten times that of the coarsest. The dissociation constant has a tendency to increase with diminishing particle size. This is true of the sub-fractions obtained from either soil.

The buffer capacity curves of the various sub-fractions obtained on plotting the buffer capacity,  $\beta$  ( $\beta = \Delta\beta/\Delta pH$ ) against the amount of the base (NaOH) added show definite maxima. In Table IX the maximum buffer capacities ( $\beta$  max.) of the various sub-fractions, as also the  $pH$  and the percentage neutralization at maximum buffer capacity, have been compared.

TABLE IX

*Maximum buffer capacity, and pH and per cent neutralization at maximum buffer capacity of hydrogen clays*

Sol	$\beta$ max.	$pH$ at $\beta$ max.	Percent neutralization at $\beta$ max.
L <sub>1</sub>	5.8	7.45	69.6
L <sub>2</sub>	11.0	7.20	62.5
L <sub>3</sub>	19.0	7.50	79.1
L	10.5	7.30	64.6
M <sub>1</sub>	5.0	6.68	65.7
M <sub>2</sub>	10.5	6.90	71.4
M <sub>3</sub>	26.0	6.70	71.2
M <sub>4</sub>	66.0	5.63	60.
M	50.0	6.45	66.0

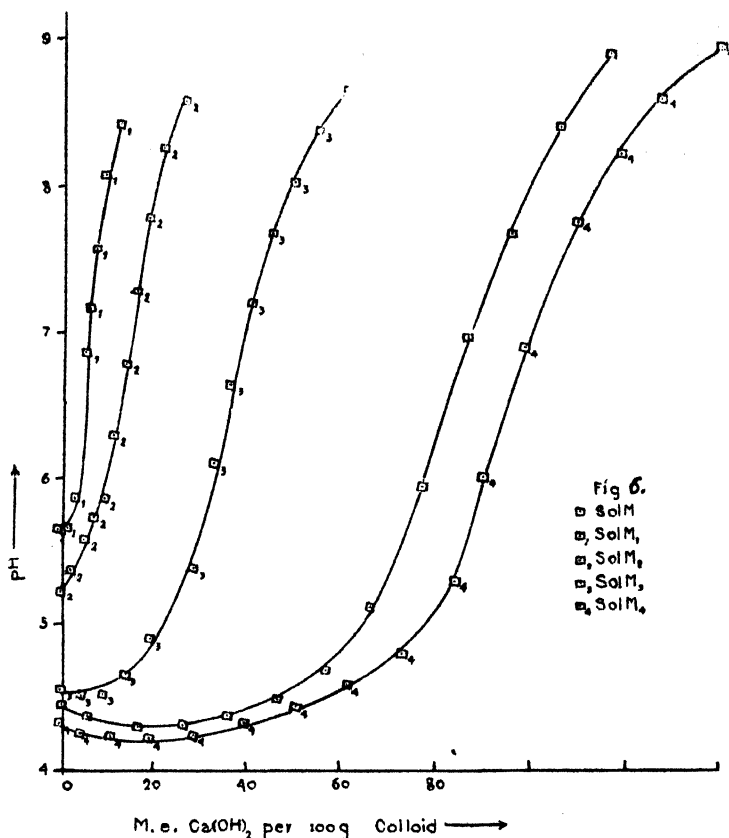


Fig. 6. Titration curves with  $\text{Ca}(\text{OH})_2$  of the entire hydrogen clay and its sub-fractions isolated from the Akola soil

The finer the fraction, the higher is  $\beta$  max. With  $L_1$ ,  $L_2$ ,  $L_3$  and  $L$ ,  $\beta$  max. occurs within a small range of  $pH$ . This is also true of  $M_1$ ,  $M_2$ ,  $M_3$  and  $M$ .  $\beta$  max. of  $M_4$ , however, occurs at a much lower  $pH$  compared with the other hydrogen clays. It is to be noted that  $\beta$  max. in no case corresponds to 50 per cent neutralization as would be expected in the case of a dissolved weak acid. It occurs at a higher stage of neutralization (60 to 80 per cent). Evidently, the interaction of the hydrogen clays with the base is not a simple neutralization of  $H^+$  by  $OH^-$  ions. It is complicated by other factors [Mukherjee, Mitra and Mukherjee, 1937.]

The  $\text{Ba}(\text{OH})_2$  curves of  $L_1$ ,  $L_2$ ,  $L_3$  and  $L$  given in Fig. 2 are not all quite similar. Those of  $L_1$  and  $L_3$  have an apparent weak acid character.  $L$  and  $L_2$  on the other hand, behave as strong acids. The dissociation constant calculated from the  $pH$  at half neutralization is  $6.7 \times 10^{-7}$  for  $L_1$  and  $5.5 \times 10^{-6}$  for  $L_3$ . The finer fraction thus behaves as a stronger acid.

The  $\text{Ca}(\text{OH})_2$  curves of  $L$ ,  $L_1$  and  $L_2$  have the same form which is different from that of sol  $L_3$  (Fig. 3).

The  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  curves of  $M_1$ ,  $M_2$ ,  $M_3$ ,  $M_4$  and  $M$  given in Figs. 5 and 6 show a general similarity of form. Those of  $M$ ,  $M_2$ ,  $M_3$  and  $M_4$  present one peculiar feature, viz. that on the first addition of the base, the

pH of the sol instead of increasing or remaining constant shows a definite decrease. This is specially marked with the baryta curves of the finer fractions. Acid appears to be liberated as a result of the interaction between the sol and the base, a result which is foreign to classical principles of acid-base interactions. An explanation of the observation is deferred pending completion of further investigations now in progress.

(d) *Total acidity per gramme and per sq. cm. of surface.*—The increase in the total acid with diminishing particle size (Table VI) may be referred to an increase in the specific surface and hence to a greater number of exchange spots in a given area. In Table X the total acids of the sub-fractions from the black soil calculated per gramme ( $T_g$ ) and per sq. cm. of the surface ( $T_s$ ) have been compared.

TABLE X

*Specific surface and b. e. c. per gm. and per sq. cm. of surface of hydrogen clays*

Hydrogen clay	Average sp. surface in sq. cm $\times 10^3$	Total acid in m. e. NaOH at inflexion point	
		per gm. ( $T_g$ )	per sq. cm $\times 10^7$ ( $T_s$ )
$M_1$	18.5	0.038	20.5
$M_2$	66.5	0.140	21.0
$M_3$	120.0	0.365	30.0
$M_4$	>160.0	0.860	<55.0

It will be seen that  $T_s$  does not show any marked or regular variation with the particle size\* though  $T_g$  rapidly increases as the latter decreases.

#### GENERAL DISCUSSION

The variations in the total acid per gramme with the particle size may arise from variations in the nature of the reactive material making up the various fractions as the differences in chemical composition would also suggest. The similarity in the form of the titration curves of the different fractions, specially the NaOH curves, and the fair agreement between the values of the dissociation constant calculated from them, on the other hand, indicate that the active acidic material present in the different fractions is essentially the same. Considered in this light the variations in the total acid (per gramme) and chemical composition may be due to varying admixtures of 'free' silica and sesquioxides having negligible base-combining capacity in the different fractions. The differences in composition might also arise from isomorphous replacements [Marshall, 1935] within the lattice of the constituent minerals and/or differences in relative proportions of several types of

\*Variations in  $T_s$  have been observed using sub-fractions from other soils. These will be discussed in the next paper of this series

clay minerals in the different fractions but these would probably give rise to more marked variations in the form of the titration curves than observed in this work.

#### SUMMARY

The variations in chemical composition, form of titration curves with bases and the base exchange capacities (b.e.c.) calculated from these curves of hydrogen clays prepared from three and four sub-fractions respectively of the entire clay fraction of a red lateritic soil from Dacca (Bengal) and a black soil from Akola (Central Provinces) have been studied. As the particle size decreases, the percentage of  $\text{Al}_2\text{O}_3$  increases but that of  $\text{SiO}_2$  and  $\text{Fe}_2\text{O}_3$  diminishes as also the silica-sesquioxide ratio. The b. e. c. and the amount of free  $\text{H}^+$  ions calculated per gramme rapidly increase with diminishing particle size but calculated per square centimetre of the external surface, the b. e. c. shows no marked or regular variation. The different sub-fractions prepared from the same soil give nearly the same type of titration curves.

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# ALTERATIONS IN THE PROPERTIES OF HYDROGEN CLAYS ON THE REMOVAL OF FREE INORGANIC OXIDES CONTAINED IN THEM, I \*

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(With five text-figures)

THE inorganic colloidal material of soil, besides containing Si, Al and Fe in a combined form making up its mineral constituents proper, is often associated with varying amounts of free oxides of these elements. The role of these free oxides in determining the base exchange and other properties of the soil colloidal material is not very well understood. A systematic study has been undertaken of the changes in properties of hydrogen clays consequent on the removal of the free oxides contained in them. The methods usually used for this purpose are not always free from the criticism that they may decompose, or, alter the properties of the clay minerals and may not effect a complete separation of the oxides. In spite of these limitations it is of interest to examine the changes brought about by such separations. The present paper deals with changes consequent on treatments according to the methods of Tamm [1922], Mattson [1931], and Drosdoff and Truog [1935] for the removal of the free oxides. The following properties have been studied : (i) chemical composition, (ii) the form of potentiometric titration curves with bases and (iii) the base exchange capacity calculated from these curves. The hydrogen clays used are listed on the next page. Further results will be reported in subsequent papers of this series.

## EXPERIMENTAL

(a) *Separation of the free inorganic oxides.*—Tamm uses a solution of acid ammonium oxalate having a pH 2.32. In Mattson's method which is used for the separation of the free sesquioxides only the clay is treated with a hot saturated solution of aluminium chloride. In the method of Drosdoff and Truog, free silica and alumina are first removed by digestion at 70°C., with 2 per cent solution of sodium carbonate. The free ferric oxide is then removed as iron sulphide which is formed on passing H<sub>2</sub>S gas through an aqueous suspension of the clay.

\*The results given in this paper have been taken from the published Annual Reports for 1935-36, 1936-37 and 1938-39 on the working of a scheme of research financed by the Imperial Council of Agricultural Research, India.

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Reference No. of hydrogen clay (before removal of free oxides)	SiO <sub>2</sub> /R <sub>2</sub> O <sub>3</sub> ra- tio of hydro- gen clay	Lab. No. and description of soil from which hydro- gen clay was obtained
F	1.94	High land acid soil from Government Farm, Burdwan (Bengal) collected at a depth of 0—6 inches from Block B, Plot No. 40. Lab. No. 14
I	2.50	Neutral calcareous soil from Satara district (Bombay) collected at a depth of 0—6 inches. Lab. No. 25
K	2.54	Neutral black soil from Bilaspur near Raipur (C. P.) collected at a depth of 0—6 inches. Lab. No. 32
L	1.99	Red lateritic soil from Government Farm at Dacca (Bengal) collected at a depth of 0—6 inches. Lab. No. 22
N	1.88	Bhata red laterite soil from C. P. collected at a depth of 0—9 inches. Lab. No. 33

(b) *Preparation of hydrogen clays*.—Hydrogen clays were prepared from the entire clay fractions (before and after removal of their free oxides) by leaching them with 0.02*N* hydrochloric acid. In the sequel, the subscripts *a*, *b* and *c* to the reference numbers of hydrogen clays have been used to denote those prepared after removal of the free oxides by the methods of Mattson, Tamm, and Drosdoff and Truog respectively.

(c) *Chemical analysis*.—The percentages of SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub> in the hydrogen clays have been determined after fusion with sodium carbonate [Wright, 1937].

(d) *Electrometric titration of hydrogen clays*.—Details of procedure adopted for this work have been described elsewhere [Mukherjee *et al.*, 1936 ; Mitra 1936, 1940]. Hydrogen and glass electrodes were used.

## RESULTS

### *A. Hydrogen clays prepared from the Burdwan Farm soil (Lab. No. 14)*

The free oxides were removed by Tamm's method. Figs. 1 and 2 give the titration curves with bases. The base exchange capacities\* (b.e.c.) calculated from the curves are given in Table I and the results of fusion analysis in Table II.

TABLE I

*Base exchange capacities of hydrogen clay from Burdwan soil before and after removal of free oxides*

Hydrogen clay	Base used for titration	pH at inflexion	B.e.c. in m.e. base per 100 gm. colloid at in- flexion point of tit- ration curve
F	NaOH . . . .	6.15 ; 7.85	9.0 ; 31.6
	Ba (OH) <sub>2</sub> . . . .	7.0	31.0
F <sub>b</sub>	NaOH . . . .	4.7 ; 7.2	3.0 ; 25.0
	Ba (OH) <sub>2</sub> . . . .	5.0 ; 6.2	7.5 ; 25.0
	Ca (OH) <sub>2</sub> . . . .	6.2	25.0

\* Reproducible to within  $\pm 2.5$  per cent

TABLE II

*Chemical composition of hydrogen clay from Burdwan soil before and after removal of free oxides*

Hydrogen clay	SiO <sub>2</sub> per cent	Al <sub>2</sub> O <sub>3</sub> per cent	Fe <sub>2</sub> O <sub>3</sub> per cent	SiO <sub>2</sub> /Al <sub>2</sub> O <sub>3</sub> (molar)	SiO <sub>2</sub> /R <sub>2</sub> O <sub>3</sub> (molar)
F . . . . .	49.2	29.2	21.4	2.85	1.94
F <sub>b</sub> . . . . .	51.5	30.9	17.4	2.82	2.01

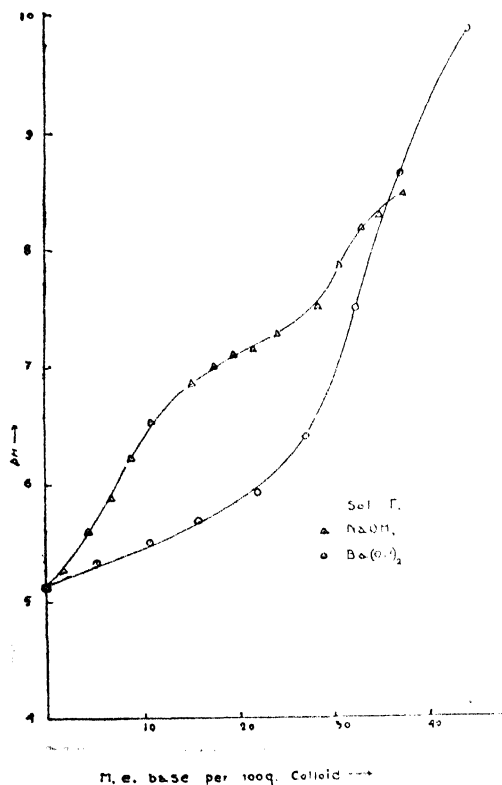


FIG. 1. Titration curves of hydrogen clay from Burdwan soil before removal of free oxides

The changes consequent on the removal of the free oxides may be summed up as follows :

1. Chemical composition . . . . .
2. Base exchange capacity . . . . .
3. Form of titration curves . . . . .

SiO<sub>2</sub> (per cent) F<sub>b</sub> > F  
Al<sub>2</sub>O<sub>3</sub> (per cent) F<sub>b</sub> > F  
Fe<sub>2</sub>O<sub>3</sub> (per cent) F<sub>b</sub> < F

F<sub>b</sub> < F

The curves of F<sub>b</sub> show a weaker initial buffer action than those of F; the Ba(OH)<sub>2</sub> curve of F<sub>b</sub> shows a dibasic acid character not observed with F

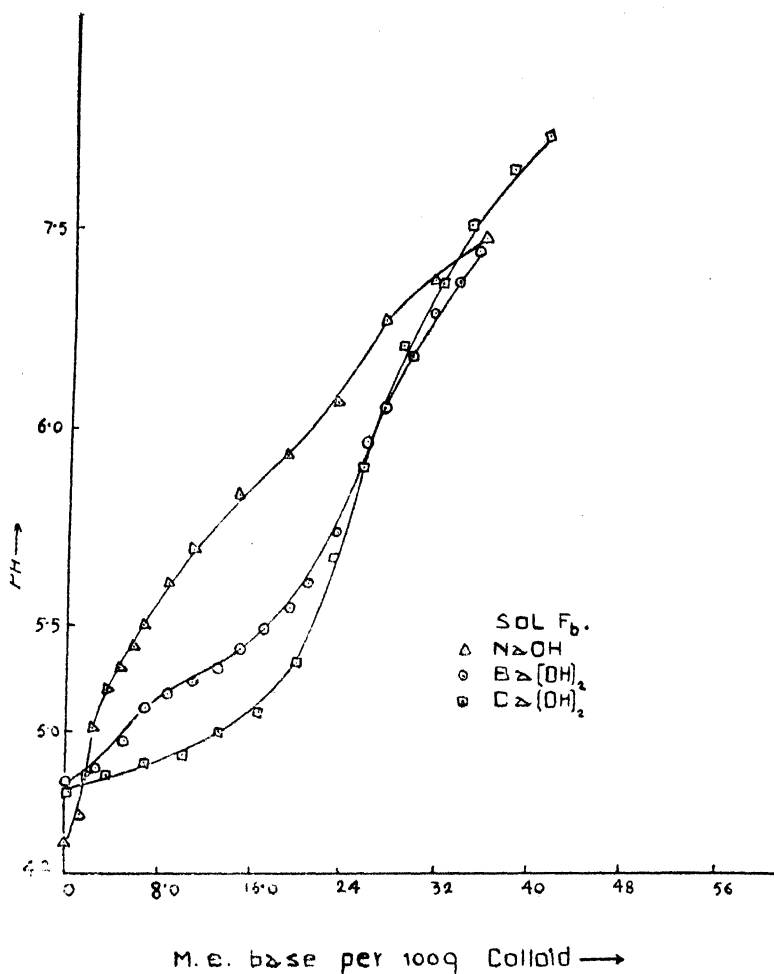


FIG. 2. Titration curves of hydrogen clay from Burdwan soil after removal of free oxides by Tamm's method

The alterations in chemical composition and the form of the titration curves point to a definite change in the hydrogen clay as a result of the treatment. The decrease in the b.e.c. suggests that this treatment brings about a decomposition of the exchange complex; an increase would have been observed if only free oxides having little or no base combining capacity had been removed.

*B. Hydrogen clays prepared from the black cotton soil from Satara (Lab. No. 25) and the black soil from Raipur (Lab. No. 32)*

The free oxides were removed by the method of Drosdoff and Truog. The base exchange capacities of I and K and their derivatives  $I_c$  and  $K_c$  calculated from the titration curves have been given in Table III and the results of fusion analysis in Table IV. All the four hydrogen clays give the same

type of titration curve with any given base. The titration curves of  $I_c$  only are given in Fig. 3.

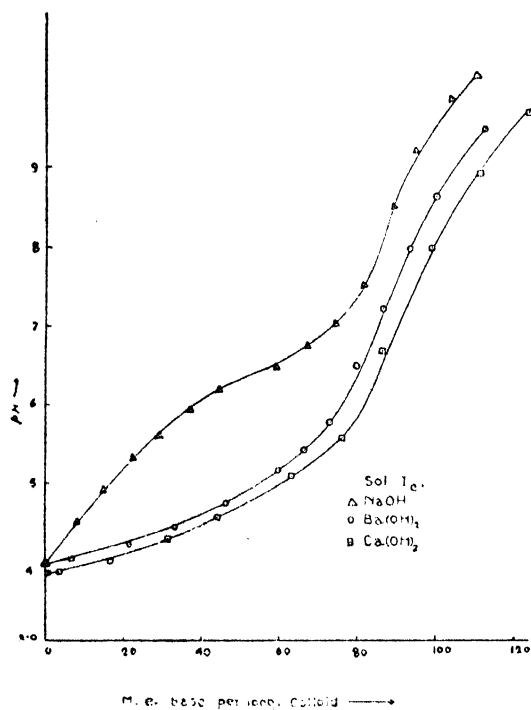


FIG. 3. Titration curves of hydrogen clay from the Satara soil after removal of free oxides by Drosdoff and Truog's method

TABLE III

*Base exchange capacities of hydrogen clays from the Satara and Raipur soils before and after removal of free oxides by Drosdoff and Truog's method*

Hydrogen clay	Base used for titration	pH at inflexion point in the titration curve	B.e.c. in m.e. base per 100 gm. oven-dried colloid	
			At inflexion point	At pH 7.0
I	NaOH	8.05	90.0	78.0
	Ba(OH) <sub>2</sub>	7.00	82.0	82.0
	Ca(OH) <sub>2</sub>	6.95	96.0	97.0
$I_c$	NaOH	8.10	86.0	74.0
	Ba(OH) <sub>2</sub>	7.60	91.0	85.0
	Ca(OH) <sub>2</sub>	6.50	86.0	91.0
K	NaOH	7.15	68.0	67.0
	Ba(OH) <sub>2</sub>	5.80	55.0	61.0
	Ca(OH) <sub>2</sub>	5.20	58.0	67.0
$K_c$	NaOH	8.10	67.5	60.0
	Ba(OH) <sub>2</sub>	5.75	56.0	62.0
	Ca(OH) <sub>2</sub>	5.78	63.0	68.0

TABLE IV

*Chemical compositions of hydrogen clays from the Satara and Raipur soils before and after removal of free oxides*

Hydrogen clay	SiO <sub>2</sub> per cent	Al <sub>2</sub> O <sub>3</sub> per cent	Fe <sub>2</sub> O <sub>3</sub> per cent	SiO <sub>2</sub> /R <sub>2</sub> O <sub>3</sub> (molar)	SiO <sub>2</sub> /Al <sub>2</sub> O <sub>3</sub> (molar)
I . . . .	55.03	24.20	20.81	2.50	3.87
Ic . . . .	55.95	24.57	18.71	2.54	3.72
K . . . .	55.93	28.03	14.80	2.54	3.40
Kc . . . .	58.30	26.90	15.80	2.66	3.65

The changes consequent on the treatment are summed up in the chart given on the next page.

If the alterations in chemical composition merely indicated a removal of free oxides which are considered to have little base combining power, an increase in the b.e.c. would be expected. The b.e.c. of the Satara hydrogen clay actually shows a slight decrease\* which probably indicates that a decomposition of the exchange complex is responsible for the variation in chemical composition. The b.e.c. of the other hydrogen clay remains practically unaltered and in order to reconcile this result with the small but definite variation in the chemical composition, it has to be assumed that the substances removed by the treatment were not 'inert'; they had, mass for mass, nearly the same b.e.c. as the original hydrogen clay.

*C. Hydrogen clays prepared from the Bhata laterite soil (Lab. No. 33) and the red lateritic soil from Dacca (Lab. No. 22)*

Laterite soils usually contain free sesquioxides. Mattson's method was therefore used in the case of the above two hydrogen clays.

The b.e.c.'s of L and N and their derivatives L<sub>a</sub> and N<sub>a</sub> are given in Table V and the results of fusion analysis in Table VI. Figs. 4 and 5 show the titration curves of L and L<sub>a</sub>. The titration curves of the other two hydrogen clays are similar to those of L and have been omitted.

\*Except for the slight increase in the b.e.c. at pH 7.0 with Ba(OH)<sub>2</sub>; this increase, however, is almost within the limits of experimental error ( $\pm 2.5$  per cent)

Hydrogen clay from	Chemical composition			B.e.c. with					Form of titration curves with bases
	SiO <sub>2</sub> per cent	Al <sub>2</sub> O <sub>3</sub> per cent	Fe <sub>2</sub> O <sub>3</sub> per cent	NaOH		Ba(OH) <sub>2</sub>		Ca(OH) <sub>2</sub>	
				At inflexion point	At pH 7.0	At inflexion point	At pH 7.0		
1. Black cot- ton soil from Satara	Increases	Increases	Decreases	Slightly de- creases	Slightly de- creases	Increases but the pH at in- flexion after removal of free oxides is definitely higher	Slightly in- creases	Decreases	Decreases
2. Black soil from Raipur	Increases	Decreases	Increases	No change though the pH at in- flexion after removal of free oxides is higher	Decreases	No change	No change	Increases but pH at in- flexion after removal of free oxides is higher	No change
									The form of the curves remains prac- tically un- altered ex- cept for variations in the pH's at the inflexion point

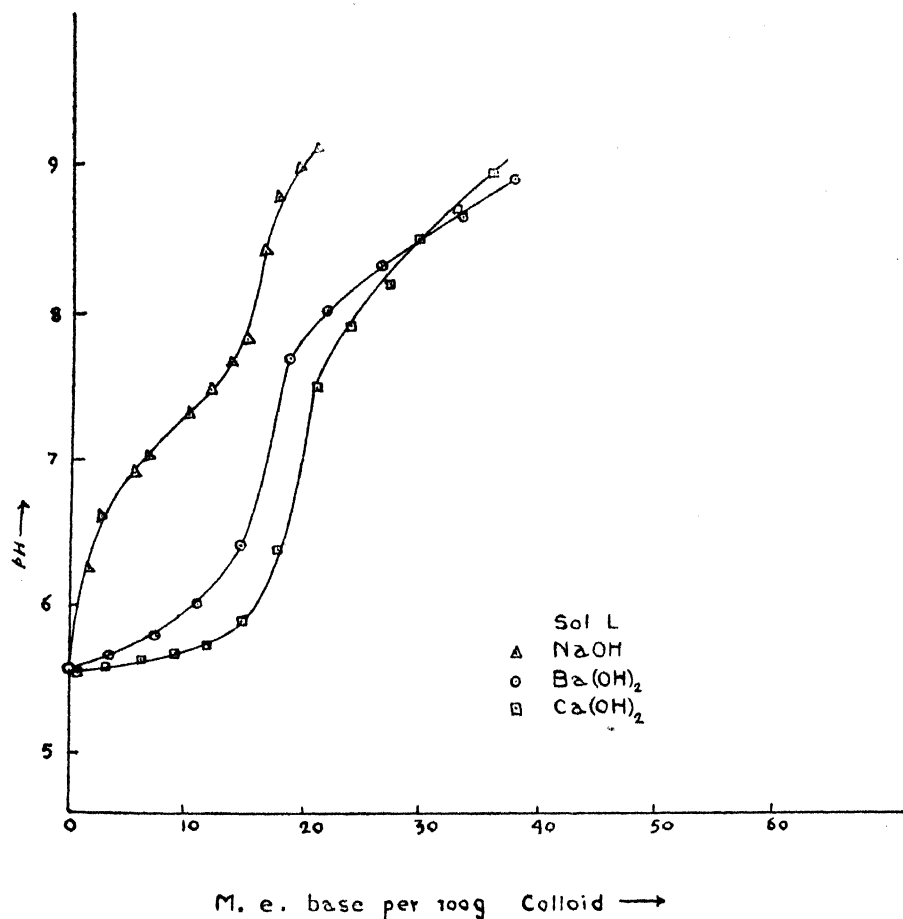


FIG. 4. Titration curves of hydrogen clay from Dacca soil before removal of free oxides

TABLE V

*B.e.c. in m.e. base per 100 gm. of hydrogen clays from Dacca and Bhata soils before and after removal of free oxides by Mattson's method*

Hydrogen clay	Base used					
	NaOH		Ba(OH) <sub>2</sub>		Ca(OH) <sub>2</sub>	
	At inflexion point	At pH 7.0	At inflexion point	At pH 7.0	At inflexion point	At pH 7.0
L . . .	16 25	6.3	17.5	17.0	19.0	19.5
L <sub>a</sub> . . .	7* ; 6.0**	6.8	8.5	9.0	11.5	11.0
N . . .	18	11.3	19.0	19.0	21.5	21.8
N <sub>a</sub> . . .	11	7.5	15.3	15.3	17.5	18.0

\*Calculated from the first inflexion point

\*\*Calculated from the second inflexion point

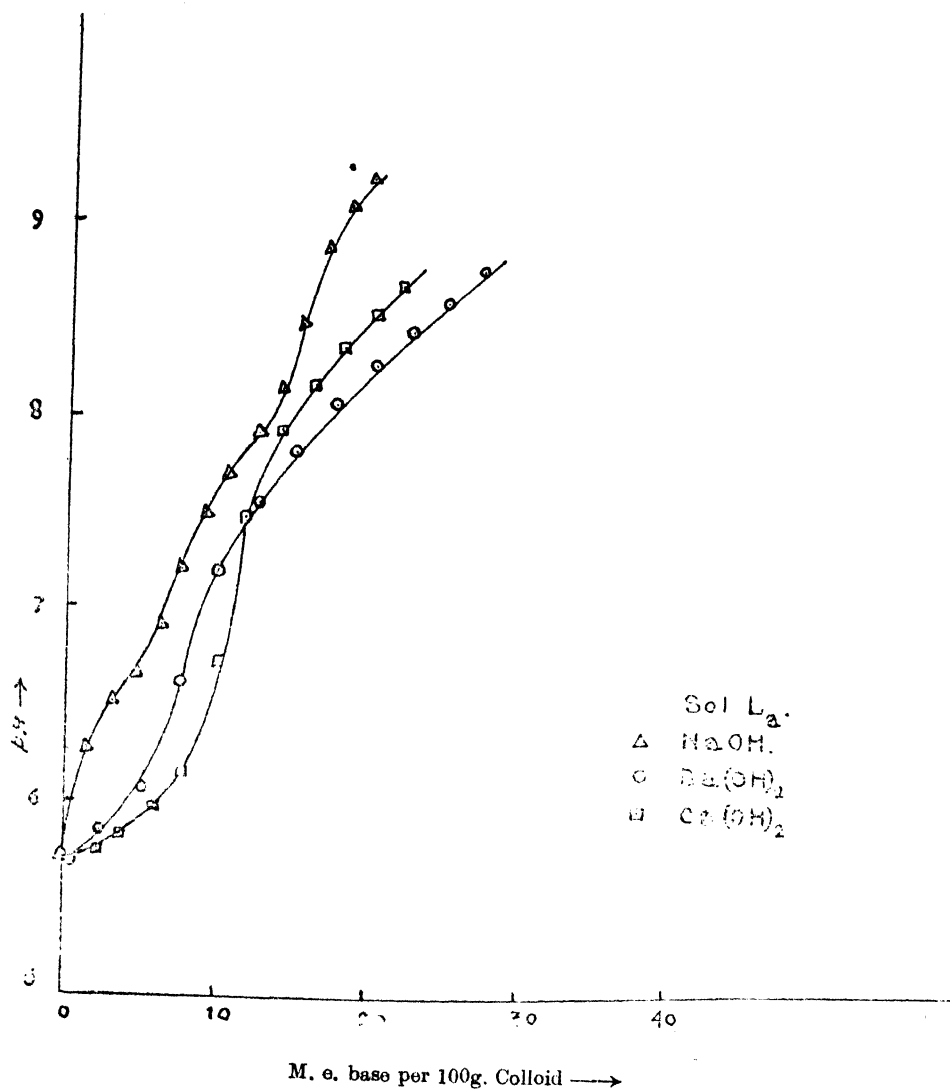


Fig. 5. Titration curves of hydrogen clay from Dacca soil after removal of free oxides by Mattson's method

TABLE VI

*Chemical compositions of hydrogen clays from Dacca and Bhata soils before and after removal of free oxides*

Hydrogen clay	SiO <sub>2</sub> (per cent)	Al <sub>2</sub> O <sub>3</sub> (per cent)	Fe <sub>2</sub> O <sub>3</sub> (per cent)
L	51.2	36.0	12.0
L <sub>a</sub>	50.0	38.8	10.1
N	42.6	3.70	54.2
N <sub>a</sub>	42.0	4.50	53.4

The percentage of silica and ferric oxide decreases as a result of the treatment, while that of alumina increases. The b.e.c. of both L and N decreases. If only free sesquioxides having negligible b.e.c. were removed, an increase in the b.e.c. would have been observed. The treatment also brings about a material change in the form of the NaOH curve of L. While L behaves as a weak monobasic acid judging from this curve, its derivative  $L_a$  shows a dibasic acid character.

Attention may finally be drawn to one general feature observed with all the hydrogen clays both before and after the treatments for the removal of their free oxides. At any given pH, the slopes of their titration curves with different bases are usually arranged in the order  $\text{NaOH} > \text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2$ , which shows that these three bases react with the hydrogen clays in the reverse order. This difference in the relative effects of the three bases illustrates what has been designated by us as an irregular or specific cation effect. It has been fully discussed elsewhere [Mitra, 1936, 1940 ; Mukherjee, Mitra and Mukherjee, 1937].

#### SUMMARY

A study has been made of the effect of treatments aiming at the removal of free silica and sesquioxides contained in hydrogen clays on their chemical composition, nature of titration curves with bases and base exchange capacities (b.e.c.). Hydrogen clays prepared from the entire clay fraction of five Indian soils have been used and the methods of Tamm, Drosdoff and Truog, and Mattson were employed for the removal of the free oxides. Tamm's method gives rise to a decrease in the b.e.c. of a hydrogen clay from a Burdwan (Bengal) soil. The chemical composition and the form of the titration curves are also materially altered indicating, on the whole, a decomposition of the exchange complex as a result of the treatment. Practically no change occurs in the b.e.c. and the nature of the titration curves of hydrogen clays from two black cotton soils using the method of Drosdoff and Truog. Slight changes in the chemical composition are, however, observed. Mattson's method gives rise to a marked decrease in the b.e.c. of hydrogen clays from a red lateritic soil from Dacca (Bengal) and a red laterite soil from the Central Provinces. The form of the titration curve with caustic soda of the hydrogen clay from the Dacca soil is also altered.

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# STUDIES IN KUMAUN HILL SOILS

## III. SOIL TYPES AT DOONAGIRI

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THE soil survey work reported by us for Chaubattia in the first two parts of this series of publication has also been extended to other parts of Kumaun, and in the present paper a consolidated account of the soil types found at Doonagiri is discussed. The technique of our survey and the methods involved have already been described in detail in the previous papers, and in the present instance only a bare outline of these will be given.

Doonagiri lies north of Chaubattia at a distance of 16 miles as the crow flies. The parent material of the soils is phyllite; granite gneiss has been found at only a few places. Although a large number of soils studied by us are clayey, due to high content of organic matter, the soils, however, are not indurated. The general forest flora of the locality is very much similar to those of Chaubattia. Average rainfall is over 60 inches a year. Situated further interior into the hills, and being a little higher, the climate of Doonagiri is slightly cooler and comparatively more humid than that of Chaubattia.

The estate was deforested in about 1867 and planted to tea. After about 20 years due to transport and other difficulties the plantation was abandoned, and no attempt has since been made to rehabilitate the area.

### LITERATURE

Information on hill soils is very scanty and the literature suffers from scarcity of data. The present position of the work on hill soils was summarized in the two foregoing papers of this series [Mukerji and Das, 1940; 1941], and no attempt has, therefore, been made here to review the literature.

The importance of the clay fraction in soil characterization was shown by Robinson [1930] for certain profiles of north Wales. Various authors have since utilized this method for the classification of soil profiles studied by them. Recently Mukerji and Das [1940; 1941] in their studies on hill soils have shown the usefulness of  $\text{SiO}_2/\text{R}_2\text{O}_3$  ratio in determining the precise characteristics of soil types in the Himalayan foot-hills in the United Provinces.

## ANALYTICAL METHODS

*Clay*

International pipette method was used for the determination of clay content of soils. After preliminary treatment with hydrogen peroxide and hydrochloric acid, dispersion was brought about by ammonia.

*Organic carbon*

Walkley and Black's method [1934] was used for this determination.

*pH*

pH values were determined by means of quinhydrone electrode.

*Sesquioxides*

For the determination of sesquioxides, HCl digestion was done according to the method of Agricultural Education Association.

*Base-saturation*

Barium acetate and ammonium chloride method of Parker [1929] was utilized for the determination of exchangeable acidity and base-exchange capacity in the same sample.

*Clay composition*

Robinson's method [Wright, 1939] was followed, and sesquioxides and silica were determined by the usual method for estimating silicates.

## DATA AND DISCUSSIONS

A large number of soil profiles were examined at Doonagiri, and in the succeeding pages a critical account of six profiles typical of the area will be given. It has been shown in the previous two papers of this series that silica-sesquioxide ratios together with the figures for percentage base-saturation give more or less an accurate idea of the local soil types. These data will, therefore, be examined for all the profiles studied along with such other relevant data as necessary.

*Pit No. 2 : Doodhatoli*

Horizon	Depth	Description
I . .	0-1 ft. 3 in.	Grey ; granular ; loamy with a slightly brownish tinge. Dark grey when wet
II . .	1 ft. 3 in.-2 ft. 3 in.	Same as above ; particles more granular ; appear to contain more organic matter than above
III . .	2 ft. 3 in.-4 ft. 6 in.	Organic ; clayey loam ; dark grey. Colour same as of the second horizon

TABLE I  
Summarized analytical data of Doonagiri soils  
(Pit No. 2)

Horizon	Per cent (air-dry basis)					C/N	pH
	Clay	Organic carbon	Total nitrogen	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>		
I	23.35	3.9	0.23	5.59	8.97	16.96	6.2
II	21.75	7.6	0.25	8.32	8.10	30.40	5.8
III	30.50	5.8	0.20	8.48	7.67	29.00	5.5

The general character of the profile is organic, and Wiesenboden characteristics are revealed in C/N ratios. Clay content of different horizons, and sesquioxide distributions are rather erratic. The composition of clay fraction and base-saturation percentages of different horizons are given in Table II.

TABLE II  
Clay analysis of Doonagiri soils  
(Pit No. 2)

Horizon	Per cent (air-dry basis)			SiO <sub>2</sub> R <sub>2</sub> O <sub>3</sub>	Exchange-able H (m. e. per cent on air-dry basis)	Per cent base-saturation
	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>			
I	44.48	19.88	15.97	2.51	4.21	75.56
II	43.90	20.93	16.77	2.35	7.25	54.69
III	43.20 <sub>4</sub>	26.23	16.37	2.00	8.70	54.69

These figures, particularly eluviation of Al<sub>2</sub>O<sub>3</sub> and constancy of Fe<sub>2</sub>O<sub>3</sub> in the profile, indicate that the third horizon is not of the same age as the first two, and can be classified as a brown forest soil having podsollic tendencies.

*Pit No. 3 : Doodhatoli*

Horizon	Depth	Description
I	0-11 in.	Grey; granular; loamy; dark grey when wet
II	11 in.-1 ft. 10 in.	Dark grey; clayey; loamy; very dark grey when wet
III	1 ft. 10 in.-4 ft.	Dark grey; granular; loamy; very dark grey when wet

TABLE III  
Summarized analytical data of Doonagiri soil  
(Pit No. 3)

Horizon	Per cent (air-dry basis)					C/N	pH
	Clay	Organic carbon	Total nitrogen	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>		
I	24.45	5.5	0.30	4.95	13.07	18.33	6.0
II	29.25	6.4	0.23	8.24	10.49	27.83	5.7
III	22.50	4.8	0.14	8.80	13.36	34.29	5.9

The predominant character of the entire profile is its organic matter content, and C/N ratio increases with depth. In this respect the pedological characters of the two profiles examined at Doodhatoli are alike. Clay content and pH values are rather erratic. Clay analysis figures of this profile and base status are given in Table IV.

TABLE IV  
Clay analysis and exchangeable bases of Doonagiri soils  
(Pit No. 3)

Horizon	Per cent (air-dry basis)			SiO <sub>2</sub> R <sub>2</sub> O <sub>3</sub>	Exchangeable H (m.e. per cent on air-dry basis)	Per cent base-saturation
	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>			
I	45.74	18.23	15.57	2.75	7.98	65.33
II	43.86	25.43	15.97	2.10	7.25	60.39
III	42.08	27.63	17.97	1.82	5.80	67.78

It follows from figures given above that the second and third horizons show some of the typical characters of the brown forest soils having Wiesenboden podsollic tendencies. The first horizon, however, does not appear to be of the same age. A few more profiles were examined in this area and in visual characters they were found to be similar to pit Nos. 2 and 3.

*Pit No. 5 : Dadoi*

Horizon	Depth	Description
I	0-10 in.	Grey ; granular ; loamy ; dark grey when wet
II	10 in.-1 ft. 5 in.	Grey with a slightly brownish tinge ; loamy ; contains more clay than the top ; more grey when wet
III	1 ft. 5 in.-3 ft.	Brownish ; granular ; clayey loam ; more brown when wet

TABLE V  
Summarized analytical data of Doonagiri soils  
(Pit No. 5)

Horizon	Per cent (air-dry basis)					C/N	pH
	Clay	Organic carbon	Total nitrogen	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>		
I	25.40	2.81	0.18	5.35	13.63	15.61	6.0
II	32.85	1.9	0.15	8.32	8.39	12.62	5.6
III	36.50	1.4	0.08	8.48	10.28	17.50	5.4

It is clear from the above description and analytical data that these profiles are heavy brown forest soils, undisturbed by terracing operations, and not very much affected by surface erosion. The composition of the clay fractions, as given in Table VI, indicates the real nature of the profile.

TABLE VI  
Clay analysis and exchangeable bases of Doonagiri soils  
(Pit No. 5)

Horizon	Per cent (air-dry basis)			SiO <sub>2</sub> R <sub>2</sub> O <sub>3</sub>	Exchangeable H (m.e. per cent air-dry basis)	Per cent base-saturation
	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>			
I	43.50	29.63	15.57	1.86	2.18	86.41
II	46.26	26.93	14.77	2.16	4.35	74.71
III	47.60	25.03	13.57	2.40	9.43	36.32

Although there is a slight indication of eluviation of silica, exchange acidity and percentage base-saturation figures clearly bring out the brown forest soil characteristics of the profile. The silicious material of the second and third horizons has sometimes been ascribed to colloidal SiO<sub>2</sub> of the parent material. It is suggested that the excess of silica in the second and third horizons of most of our soils is due to capillary ascendance of silica soil from the lower layers.

*Pit No. 7: Khalkhet*

Horizon	Depth	Description
I	0-6 in.	Grey; granular; loamy sand with a slightly brownish tinge with some clay; dark grey when wet
II	6 in.-1 ft. 6 in.	Same as above, but contains more clay
III	1 ft. 6 in.-2 ft. 6 in. and below.	Brownish grey; more granular, heavy loam

TABLE VII  
Summarized analytical data on Doonagiri soils  
(Pit No. 7)

Horizon	Per cent (air-dry basis)					C/N	pH
	Clay	Organic carbon	Total nitrogen	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>		
I	13.25	6.4	0.22	7.19	7.23	29.09	6.2
II	37.50	4.2	0.15	8.88	8.29	28.00	6.0
III	35.25	4.6	0.14	10.08	9.19	32.86	5.8

The first 6 in. of the profile have been impoverished of its finer material presumably by erosion. In all other essential characters, these profiles resemble brown forest soils. The essential brown forest characters of the profile are brought out more clearly from the clay analysis figures given in Table VIII.

TABLE VIII  
Clay analysis and exchangeable bases of Doonagiri soils  
(Pit No. 7)

Horizon	Per cent (air-dry basis)			SiO <sub>2</sub> R <sub>2</sub> O <sub>3</sub>	Exchangeable H (m. e. per cent on air-dry basis)	Per cent base-saturation
	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>			
I	47.22	27.43	14.37	2.20	5.08	70.15
II	45.16	28.03	15.17	2.03	4.35	72.98
III	44.90	28.43	14.77	2.01	7.25	59.28

Whereas the character and composition of exchangeable bases resemble those of brown forest soils, a slight podsollic tendency is indicated by the drop in SiO<sub>2</sub>/R<sub>2</sub>O<sub>3</sub> ratios.

Pit No. 6 : Khalkhet (Lower level of southern highlands)

Horizon	Depth	Description
I	0-6 in. . . . .	Greyish brown ; loam containing undecomposed organic matter ; more brown when wet
II	6 in.-1 ft. 5 in. . . . .	Yellowish brown ; heavy loam ; more brown when wet
III	1 ft. 5 in.-2 ft. 6 in. . . . .	Reddish brown ; heavy loam ; deep red when wet

TABLE IX  
*Summarized analytical data on Doonagiri soils*  
 (Pit No. 7)

Horizon	Per cent (air-dry basis)					C/N	pH
	Clay	Organic carbon	Total nitrogen	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>		
I	28.95	2.7	0.13	4.67	17.23	20.85	6.4
II	32.75	1.0	0.07	12.48	10.24	14.29	5.8
III	32.75	0.8	0.05	14.96	14.46	16.00	5.7

There is quite an appreciable eluviation of clay; pH values and organic carbon percentages resemble those of brown forest soils. We find, however, considerable translocation of ferric oxide, which, as is well known, is a podsollic character.

The results of the clay analysis and data on exchangeable bases are presented in Table X.

TABLE X  
*Clay analysis and exchangeable bases of Doonagiri soils*  
 (Pit No. 6)

Horizon	Per cent (air-dry basis)			SiO <sub>2</sub> R <sub>2</sub> O <sub>3</sub>	Exchangeable H (m.e. per cent on air-dry basis)	Per cent base-saturation
	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>			
I	44.04	26.83	16.77	1.99	8.70	55.84
II	44.60	24.03	15.97	2.21	1.45	87.50
III	40.62	21.34	20.76	1.99	1.45	89.10

The data given above leave no doubt as to the genetic nature of these profiles. There is a very high eluviation of Fe<sub>2</sub>O<sub>3</sub>, specially from the first to the third horizon. The base-exchange figures also indicate podsollic tendency in the profile under reference. In view of the constancy of composition of SiO<sub>2</sub>/R<sub>2</sub>O<sub>3</sub> ratios this profile may be classified as brown forest soil with podsollic tendency.

The general characters of all the five profiles discussed above show the existence of one genetic type of soil formation in this area. A casual observation, however, of the hill slopes in this area as well as over the whole of Kumaun

shows the presence of a large variety of soils with colours varying from deep red to yellow. These soils are often shallow and occupy quite an appreciable area of the Kumaun hills, and are utilized for the growing of grain crops or fruit trees. A number of profiles were examined in this area and one such profile is described under and summarized analytical data are presented in Table XI.

*Pit No. 1 : Kiyari (Western ridge)*

Horizon	Depth	Description
I	0-1 ft. 6 in.	Yellowish brown; loamy sand; more brown when wet and sticky. Some decomposing platy phyllite rock present

TABLE XI  
*Summarized analytical data of Doonagiri soils*  
(Pit No. 1)

Per cent (air-dry basis)					C/N	pH
Clay	Organic carbon	Total nitrogen	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>		
16.45	0.86	0.03	8.70	20.50	28.66	5.6

Absence of organic matter and therefore the seat of eluvial activity is clearly indicated by both visual observations and by low contents of organic carbon and total nitrogen. The figure for the sesquioxides is comparatively higher and pH value much lower than what is usual for eluvial horizon in this locality.

Data on clay composition and exchangeable bases are given in Table XII.

TABLE XII  
*Clay composition and exchangeable bases of Doonagiri soils*  
(Pit No. 1)

Per cent (air-dry basis)				Exchangeable H (m. e. per cent on air-dry basis)	Per cent base-saturation
SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	$\frac{\text{SiO}_2}{\text{R}_2\text{O}_3}$		
45.02	20.47	20.73	2.27	4.99	62.48

It is clear from the data presented in Tables XI and XII that the profile has some essential characters of lower horizons of pure brown forest soils discussed earlier in this section. The predisposing cause leading to the development of these soils is intense surface erosion in the past, but often such conditions are also brought about by faulty terracing practices. The soils because of their acidic nature and peculiar dynamics should be classified as a variety of brown forest soils resembling truncated forest soils of Sigmond's [1938] main type 4 of soil order 10. Other profiles studied in this area have characteristics similar to those described in these pages and can be classified into two major genetic groups, viz. brown forest soils and brown forest soils with podsollic tendency.

### DISCUSSION

In the first two parts of this series of papers the possibility of classifying hill soils according to their developmental characteristics has been clearly indicated. In the present instance the same method of soil classification has been followed. It has been possible by this method in the present case not only to understand the characteristics of hill soils as a class but also the genesis of the individual profiles. It is interesting to note that silica/sesquioxide ratios of the clay fractions and per cent base-saturation figures taken together supply all the important data necessary for the classification and characterization of soils in the hills. It is not implied by this that the organic matter content, pH and mechanical composition do not offer any valuable assistance in the studies of hill soils, but that such determinations have limited importance in consequence of the fact that they only confirm the findings arrived at from a study of the clay composition and exchangeable bases.

Doonagiri soils belong to the usual brown forest soil type and on the average these soils are very much richer in organic matter. Moreover, the soils of this locality contain a high proportion of plant nutrients. The largest number of successful fruit orchards of these hills are situated on soils formed from phyllite rock like that of Doonagiri, a fact that cannot be ascribed to chance alone.

The prevalent colours of most of these soils at Doonagiri are black and reddish brown. Soils having the latter colour, as has been indicated above, belong to truncated brown forest soil type. Soils having black colour are an important study by themselves. It is not, as we find, that the higher content of organic carbon always accounts for the black colour of the soil, but a high C/N ratio seems to have some direct bearing on the black colouration. Such seems to have been the case with pit No. 2. Soil containing 7.6 per cent of organic carbon appears to be as organic as another soil which contains only 5.8 per cent organic carbon. Pit No. 3 also shows similar characteristics. Profile No. X15 Y15 [Mukerji and Das, 1941] is an analogous case.

The mean  $\text{SiO}_2/\text{R}_2\text{O}_3$  ratio in the clay fractions of these profiles is  $2.15 \pm 0.062$ . This ratio is well within limits to be consistent with the hypothesis that the primary weathering product of this locality is a mixture of hydrated silicates having the general formula  $\text{R}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot x\text{H}_2\text{O}$ . Deviations from absolute value of 2 may have been brought about by eluviation and translocation. The usefulness of the silica/sesquioxide and silica/

alumina ratios for determining with certainty the genetic characteristics of hill soils will be discussed more fully in the subsequent parts of this series of publications dealing with the developmental characters of soils derived from other rock materials.

#### SUMMARY

The nature of some soils formed at Doonagiri has been discussed.

Clay composition and per cent base-saturation taken together have been found very useful in characterizing these soils.

Silica/sesquioxide ratio has been found to be  $2.15 \pm 0.24$  and, therefore, the primary weathering product has been suggested to be a clay having the general composition  $R_2O_3 \cdot 2SiO_2 \cdot xH_2O$ .

The soils of the locality contain large proportions of organic matter, and surface soils are on the whole highly base-saturated.

From the general study of the data it is evident that these soils belong to brown forest soil group.

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# STUDIES IN INDIAN RED SOILS

## VI. DETERMINATION OF MINERALOGICAL COMPOSITION

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(With one text-figure)

THE usual method of characterizing soil types in the laboratory is to find out the physical and chemical composition of the soil samples, as also of the clay fractions. These determinations, however, require long time to be carried out. Comparatively little attention has been paid, so far, to the determination of the mineral compositions of soils, at any rate in India. The presence of great variety of minerals in soils was noted by McCaughey and Fry [1913], who reported the results of mineralogical studies on the chief soil groups of the United States. They identified optically 34 different minerals in a great variety of soils and concluded that the mineralogical composition of soils varies with the physiographic regions in which they occur. Among other workers who have discussed the constitution of soils from mineralogical standpoint are Delage and Lagatu [1904 ; 1905], Cayeau [1905], Hendrick and Newlands [1923; 1925 ; 1928], Hart [1935], Marshall [1935 ; 1936], Pearson and Truog [1937] and Bonnett [1939]. The determination of soil minerals is useful to the study of soils in two different ways :—

(1) In the first place, the content of minerals may indicate the nature of the parent material of the soil.

(2) In the second place, the determination of soil minerals may indicate the nature of inorganic soil colloids and of plant foods present in the soil. Moreover, permanence of fertility in a soil varies with its contents of minerals which are still liable to decomposition. A soil having a high content of such minerals is, other things being equal, better than a soil with no mineral reserves [Vageler, 1933].

In connection with his study on the nature of colloidal minerals of clay, Nagelschmidt [1939] has discussed all the important existing methods for determining the nature of clay minerals, viz. X-ray, optical, dehydration and chemical methods with special regard to their limitations. On the other hand, the importance of determining minerals of microscopic dimensions by the petrological methods has been pointed out by various workers (for a survey of literature see Harrison [1933]). In view of the scanty data on the mineralogical composition of Indian soils, it was felt desirable to determine the mineralogical composition of fine sand fractions of some typical profile

samples of red soils collected from different parts of India. The composition with respect to the rock-forming minerals of the soil is well represented by the fine sand fraction, except for minerals with flaky structure, such as mica, which tend to accumulate in the finer fractions. Volk [1933], in studying the formation of muscovite in soils, reports quantitative separation of mineral groups by means of liquids of different specific gravities. In recent papers, Truog and coworkers [1937-38] have sub-divided mineralogical constituent of soil by means of specific gravity separation with heavy liquid, such as tetra-bromo-ethane and nitro-benzene mixtures. Hendrick and Newlands [1923] have obtained good results on the separation of the heavy and light fractions of fine sand by bromoform (sp. gr. 2.9). Also by using a mixture of bromoform and benzene (sp. gr. 2.65), they separated quartz particles from the felspar grains, the sp. gr. of the quartz particles being higher than 2.65 (middle fraction), whilst that of the felspar particles being less (light fraction).

### EXPERIMENTAL

The method of separation of the heavy, middle and light fractions of the fine sand, for the mineralogical and microscopical examination was essentially that described by Hendrick and Newlands [1923]. The work in the present paper can be divided into two sections. In the initial stages of the work (§I) the fine sand fractions with which the microscopical determinations were carried out, were not estimated quantitatively for the percentages of heavy, middle and light fractions. At this stage of the work, the heavy and light fractions were separated by shaking with bromoform (sp. gr. 2.9) only, but the separated fractions were not weighed. Also the nature of minerals on the microscopic slides were only qualitatively determined, but they were not counted for determining the percentages of different minerals in the slides.

In the second stage of the work (§II) it was felt desirable to obtain the mineralogical data of the soil samples on a quantitative basis. The fine sand fractions were accordingly separated into three fractions (heavy, middle and light) and their composition separately determined with the help of petrological microscope. The percentages of different minerals present in the slide were also carefully counted with the help of a graduated cross-wire eye-piece micrometer scale.

#### *Separation of fine sand into heavy and light fractions*

A special separating funnel illustrated in Fig. 1 was used to facilitate the work and minimize the chances of admixture of the separated minerals. The funnel is provided with a stopper closed at one end. The separated heavy mineral, therefore, collects in the opening so that by turning out the stopper the separation is complete. For actual experiment approximately 1.5-2 gm. of fine sand, after ignition, were treated with approximately 20 c.c. of N/10 oxalic acid and the mixture kept in contact for about 16 hours. By this treatment the grain were cleared of ferruginous matter. The supernatant liquid was then thrown out and the residue washed 10 times with distilled water by decantation in a beaker. The substance was then dried and an

accurately weighed portion of it was treated with sufficient quantity of bromoform in the special separating funnel described before. It was necessary for the bromoform and the substance to remain in contact with each other for a period of not less than three to four hours. The heavy fraction was then isolated by turning out the stopper and was transferred to a dry filter paper, washed with benzene and dried (heavy fraction). The light fraction which was floating on the surface of the bromoform was then filtered through a dry filter paper, washed with benzene and dried. This dried substance was subsequently treated in the same separating funnel with a mixture of bromoform and benzene of sp. gr. 2.62. The fraction which settled at the bottom of the separating funnel and that which floated on the surface of the liquid were collected separately on dried filter papers, washed with benzene and dried (middle and light fractions).

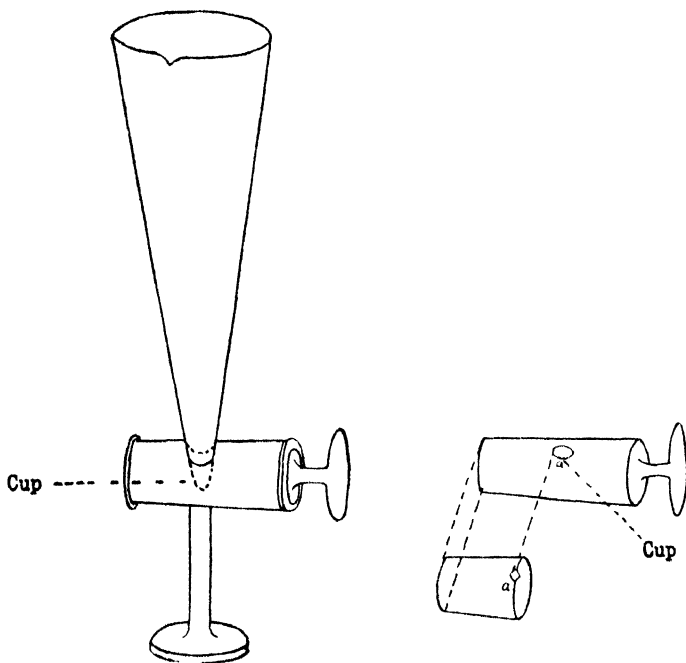


FIG. 1. Spaeth's separating apparatus

(The sample and liquid are placed in the funnel and stirred. The heavy residue collects in a cup in the tap: the turning of the tap isolates the residue until the light minerals have been removed)

#### *Preparation of microscopic slides*

The separated fine sand fractions were mounted on microscopic slides with Canada balsam by following essentially the procedure described by Milner [1932].

### §I. RESULTS AND DISCUSSION

The results have been summarized in Table I and, as far as possible, the minerals have been written in descending order of abundance. It will be found that iron ore minerals (e.g. limonite, magnetite, and haematite), as

also quartz and felspar grains, are uniformly present in high concentrations in all the profiles.

TABLE I

*Soil minerals at different localities and the geological formations of those localities*

Locality	Minerals	Parent materials*
Dacca Farm, Bengal	Iron ore minerals, quartz and felspar grains, epidote, hornblende, zircon and chloritic minerals	Old alluvium + ?
Suri, Birbhum, Bengal	Iron ore minerals, quartz and felspar grains, epidote, zircon, garnet and chloritic minerals	Recent deposit + ?
Bidar, Hyderabad	Iron ore minerals, quartz and felspar grains, garnet, chloritic minerals, epidote and biotites	Basalt + ?
Himayethsagar, Hyderabad, Deccan	Iron ore minerals, quartz and felspar grains, hornblende, epidote, augite and chloritic minerals	Granite + ?
Telankheri, Nag- pur, C. P.	Iron ore minerals, quartz and felspar grains, epidote, zircon and chlorites and occasionally augites and hornblende. Also a few biotites	Basalt + ?
Chandkhauri Farm, Raipur, C. P.	Iron ore minerals, quartz and felspar grains, garnet, zircon, chloritic minerals	Limestone + ?
Alisagar, Hydera- bad	Iron ore minerals, quartz and felspar grains, epidote, garnet, augites and a few chloritic minerals	Quartzite + ?
Kakat, Cannanore, Malabar	Iron ore minerals, quartz and felspar grains, epidote, zircon, staurolite and chloritic minerals	Granite + ?
Puzathi, Canna- nore, Malabar	Iron ore minerals, quartz and felspar grains, epidote, augite, zircon and chlorites, occasionally tourmalines	Granite + ?
Nilgiri Hills, Madras pro- vince	Iron ore minerals, quartz and felspar grains, epidote, augite, garnet, chlorite, zircon and biotite	Charnolite + ?
Gorantla Hills, Guntur, Madras	Iron ore minerals, quartz and felspar grains, epidote, zircon, garnet, hornblende and a few staurolites	Granite + ?
Stambhalaguruva, Guntur, Madras	Iron ore minerals, quartz and felspar grains, epidote, zircon, garnet, staurolites and chlorite, occasionally a few topaz	Granite + ?
Khodappanam Kunnu, Trivandrum, Travancore	Iron ore minerals, quartz and felspar grains, epidote, zircon, garnet, chloritic minerals and a few muscovites	Gneiss + ?
Government Fruit Farm, Cape Comorin	Iron ore minerals, quartz and felspar grains, epidote, hornblende, zircon, tourmaline and chloritic minerals	Gneiss + ?

\* It is likely that the exposed rocky pieces, which were collected from the places where the profiles were taken, constitute a part of the parent material. Hence the sign ' ? ' has been added.

Many of the potash feldspars of smaller sizes were turbid with decomposition products (kaolinization). In almost all the soils the quartz particles are characterized by rough angular appearance. Rounded grains are also sometimes met with. Table I shows that the common ferro-magnesian rock-forming minerals are represented by one or more species in all the soils examined, one or other being present in preponderating amount. Hornblende was present in some cases and that too in small amounts. Chlorite, an alteration product from the ferro-magnesian rocks was found in most of the soils studied. Biotite was occasionally found (e.g. Nilgiri hills). These minerals are of resistant nature and they are concentrated in material which has been subjected to prolonged weathering and has accumulated from a variety of sources. Epidote and garnet were frequently found in granular form, whilst tourmaline and especially zircon, though sometimes fragmentary, showed crystalline forms.

Iron ore minerals like haematite, limonite and magnetite were present in all the soils examined. With regard to the percentage of the ferro-silicate group of minerals in various soil samples, the soils of basic igneous origin were found to be the richest. In almost all cases the proportion of resistant minerals like epidote, garnet, zircon and tourmaline is higher compared with the common rock-forming minerals like biotite and hornblende. The latter minerals are the potential sources of plant food supply and these are usually present in the soils in small amounts, which explains the comparatively little unproductivity of these soil types. In a recent paper, Bonnett [1939] has determined the mineralogical constituents of the silt fractions of samples from a lateritic soil profile of Puerto Rico and has shown that the constituent minerals are hydrated iron oxides, gibbsite, muscovite, secondary quartz and serpentine. The accessory minerals, on the other hand, are : rutile, augite, leucoxene, zircon, magnetite, chlorite, sericite, calcite, glass, andesine, a kaolin-like mineral and traces of talc and epidote. The lateritic soils of India studied differ from those of Puerto Rico in that they are mostly found to occur on quartzites and granitic rocks except in the case of central and western and north-eastern parts of peninsular India, where the parent material is basalt. The parent material of soil type of Puerto Rico is Andesitic Tuff. The quartz particles found in such soil types seem to be mainly secondary quartz.

The presence in almost all the soil types of a large proportion of iron oxides and highly resistant unweathered minerals such as epidote, zircon, tourmaline, etc. suggests that the soil has been formed of prolonged chemical action which took place prior to present soil-forming processes. The alternative suggestion is that these soils have been subjected to extensive erosion and minerals of comparatively low specific gravity have been washed down leaving behind minerals of comparatively high specific gravity like epidote, zircon, tourmaline, etc.

## § II. MECHANICAL ANALYSES OF SOIL SAMPLES

The mechanical analyses were carried out by following essentially the procedure developed by Robinson [1933]. The data on the percentages of coarse sand, fine sand, silt and clay are shown in Table II.

**TABLE II**  
*Mechanical composition of soil samples*  
 (Air-dry basis)

Locality	Sample No.	Depth	Coarse sand (per cent)	Fine sand (per cent)	Silt (per cent)	Clay (per cent)
Hathwara, Bihar	Manbhum,	81p 0—1 ft. 6 in. . .	38·0	21·0	9·0	30·1
		82p 1 ft. 6 in.—2 ft. 3 in. .	41·0	17·5	10·8	28·6
		83p 2 ft. 3 in.—3 ft. 6 in. .	34·9	21·6	10·8	31·2
		84p 3 ft. 6 in.—4 ft. 11 in. .	34·9	11·8	15·9	33·6
		85p Below 4 ft. 11 in. .	40·0	20·0	11·2	28·6
Putida, Singhbhum, Bihar		86p Below 30 ft. .	61·6	17·1	5·5	14·7
		87p 0—1 ft. . .	22·7	21·5	17·2	37·7
		88p 1 ft.—2 ft. 9 in. .	30·9	12·0	13·2	37·0
Ratu, Ranchi, Bihar		89p 2 ft. 9 in.—4 ft. .	48·8	15·5	10·4	19·6
		90p 0—1 ft. . .	23·8	19·6	14·9	37·1
		91p 1 ft.—2 ft. . .	17·9	14·5	15·4	48·2
Baralota, Bihar	Daltonganj,	92p 2 ft.—3 ft. . .	26·0	13·0	13·2	44·5
		93p 3 ft.—4 ft. . .	31·3	11·3	9·1	45·8
		94p 0—1 ft. 11 in. . .	21·4	32·6	16·0	28·8
Tangi, Cuttuck, Orissa		95p 1 ft. 11 in.—2 ft. 9 in. .	15·7	21·1	13·8	44·5
		96p 2 ft. 9 in.—4 ft. .	16·9	16·0	12·6	49·0
		97p 4 ft.—5 ft. . .	48·8	17·1	19·6	12·1
Dhanmandal, Cuttuck, Orissa		98p 0—1 ft. . .	35·0	33·8	14·8	14·6
		99p 1 ft.—2 ft. . .	32·1	27·9	17·0	20·0
		100p 2 ft.—4 ft. . .	49·1	20·1	9·1	17·6
Kapileswar, Orissa	Bhubaneswar,	101p 0—5 in. . .	28·7	23·8	14·6	31·0
		102p 5 in.—4 ft. . .	16·0	9·0	7·5	61·5
		103p 0—2 ft. 11 in. . .	40·1	21·0	11·6	26·4
Jhinkartangi, Khurda Town, Orissa		104p 2 ft. 11 in.—4 ft. .	49·0	19·4	3·1	27·6
		105p Below 30 ft. . .	41·3	15·7	12·6	28·0
		106p 0—1 ft. . .	34·3	10·2	12·2	39·4
Lalgarh, Midnapur, Bengal		107p 1 ft.—2 ft. . .	22·1	11·0	13·4	48·9
		108p 2 ft.—8 ft. 6 in. .	44·7	11·7	11·7	28·3
		109p 8 ft. 6 in.—10 ft. .	42·5	24·9	6·7	22·4
Malida, Midnapur, Bengal		110p 30 ft.—50 ft. . .	29·9	20·5	19·0	29·3
		112p 0—4 in. . .	28·3	18·8	10·4	39·0
		113p 4 in.—3 ft. 4 in. .	40·0	18·5	2·8	35·2
		114p 3 ft. 4 in.—4 ft. .	46·6	18·2	7·6	26·7
		115p 7 ft.—8 ft. . .	13·4	41·5	6·6	34·2
		116p 0—8 in. . .	39·3	44·7	4·4	9·5
		117p Bed soil of Cossye B. .	5·0	19·3	24·3	44·0
		118p 40 ft. below . .	39·5	13·4	13·2	32·8

TABLE II—*contd.*

Locality	Sample No.	Depth	Coarse sand (per cent)	Fine sand (per cent)	Silt (per cent)	Clay (per cent)
Mawphlang, Khasi Hills, Assam	119p	0—6 in.	17.9	12.0	23.7	37.0
	120p	6 in.—1 ft. 3 in.	20.5	11.3	23.3	35.6
	121p	1 ft. 3 in.—2 ft. 1 in.	18.6	11.7	23.3	38.6
	122p	2 ft. 1 in.—4 ft.	25.0	9.5	23.4	35.4
	144p	0—3 in.	43.5	18.4	14.7	20.2
Upper Chandmari, Garo Hills, Assam	145p	3 in.—1 ft. 8 in.	54.6	18.6	10.5	15.0
	146p	1 ft. 8 in.—2 ft. 8 in.	65.2	16.4	8.4	9.1
	147p	2 ft. 8 in.—4 ft.	63.7	12.9	13.8	8.1

The mechanical analyses data show generally a more sandy texture for the surface soil than for the subsoil. This fact has been usually ascribed to sandy wash of external origin, or to mechanical eluviation within the profile. Robinson [1936] has suggested a third possibility in that it might be due to normal erosion, as distinct from catastrophic erosion, involving lateral movement of the finer fractions. Such removal, according to him, might take place along the surface of the soil itself or along the surface of a water-table and, in either case, the result would be to produce a surface horizon relatively richer in the coarser fractions than the parent material.

*Estimation of minerals of different grades in the fine sand fraction\**

The fine sand fractions were treated with oxalic acid to remove the cementing iron oxide material, washed, dried and then separated into three fractions, as detailed below, by shaking with bromoform (sp. gr. 2.9) and a mixture of bromoform and benzene (sp. gr. 2.62) :—

- (a) Sp. gr. higher than 2.9, mostly ferro-magnesian minerals
- (b) Sp. gr. between 2.62 and 2.9, mostly quartz grains
- (c) Sp. gr. less than 2.62, mostly felspar grains

All these three fractions were washed, dried and separately weighed in chemical balances. This gave the percentages of different fractions in the fine sand.

Table III shows the quantities of different fractions expressed as percentage of fine sand and of the soils.

The results show that the soils of Midnapur, Bengal, are the richest in the percentages of heavy minerals. The soils of Palamau (Daltonganj), of Cuttuck (Orissa), and of Puri (Orissa) are intermediate in their content of heavy minerals. The greater the percentages of heavy minerals present in the soil, the greater would be the probability that the soil is changing into a new one provided external disturbing factors like erosion be not present. These considerations, however, do not apply to the case when resistant minerals like garnet or iron oxide are present. On the basis of these general considerations we should expect that the soils obtained from Khasi hills, Assam, as also from the districts of Manbhum and Singhbhum have been weathered to a great extent, whilst the soil of Midnapur are comparatively immature.

\*The silt fraction was not analysed quantitatively, because it was not found practicable to utilize the heavy liquid method of separation for material of so fine a grade.

The percentages of the middle and light fractions in the soil types do not yield any useful information unless the percentages of minerals containing useful plant nutrients be separately determined.

TABLE III  
*Different fractions expressed as percentage of fine sand and of soil*

Locality	Soil No.	Heavy fraction		Middle fraction		Light fraction		Error* as per cent of fine sand
		Fine sand (per cent)	Soil (per cent)	Fine sand (per cent)	Soil (per cent)	Fine sand (per cent)	Soil (per cent)	
Hathwara Farm, Manbhum, Bihar	81p	1.2	0.252	2.1	0.440	90.4	19.0	6.3
	82p	1.8	0.315	1.7	0.298	88.4	15.5	8.1
	83p	2.2	0.475	2.2	0.475	88.4	19.1	7.2
	84p	2.2	0.26	2.4	0.284	85.2	10.1	10.2
	85p	0.5	0.1	3.3	0.66	89.2	17.84	7
	86p	7.8	1.37	37.0	6.5	48.8	8.35	5.5
Putida, Singhbhum, Bihar	87p	1.3	0.279	70.2	15.1	16.7	3.59	11.8
	88p	0.8	0.096	45.9	5.51	47.8	5.73	5.5
	89p	0.3	0.0465	60.2	9.33	32.6	5.05	6.9
Ratu, Ranchi, Bihar	90p	5.9	1.16	68.0	13.3	17.4	3.4	8.7
	91p	3.7	0.536	40.4	5.86	47.2	6.84	8.7
	92p	5.2	0.676	13.4	1.74	70.7	9.19	10.7
	93p	6.8	0.768	44.8	5.06	43.2	4.88	5.2
Daltonganj, Palamau, Bihar	94p	9.9	3.23	7.3	2.38	76.6	24.9	6.3
	95p	4.7	0.992	6.6	1.39	82.5	17.4	6.2
	96p	5.5	0.88	15.4	2.47	72.6	11.6	6.5
	97p	0.3	0.513	1.3	0.222	95.5	16.3	2.9
Tangi, Cuttuck, Orissa	98p	7.0	0.237	6.1	2.06	83.9	28.4	3.0
	99p	7.5	0.209	7.7	2.15	79.8	22.3	5.0
	100p	30.7	6.07	31.3	6.29	34.1	6.85	3.9
Dhanmandal, Cuttuck, Orissa	101p	7.6	1.81	11.9	2.83	76.7	18.3	3.8
	102p	10.8	0.972	7.1	0.639	77.4	6.60	4.7
Kapileswar, Bhubaneswar, Orissa	103p	9.2	1.93	37.7	7.91	49.2	10.3	3.9
	104p	12.8	2.48	33.9	6.58	51.5	10.0	1.8
	105p	5.4	0.848	14.9	2.33	77.6	12.2	2.1
Jhinkartangi, Khurda, Puri, Orissa	106p	9.3	0.948	32.6	3.33	54.1	5.52	4.0
	107p	12.8	1.41	36.2	3.98	44.8	4.94	6.2
	108p	16.3	1.91	20.6	3.04	58.2	6.81	4.9
	109p	25.1	6.24	45.2	11.25	26.5	6.60	3.2
Lalgah, Midnapur, Bengal	110p	2.4	4.92	19.6	4.90	74.6	15.3	3.4
	112p	2.9	5.45	1.9	0.357	92.6	17.4	2.6
	113p	2.1	3.89	8.2	1.52	87.1	16.1	2.6
	114p	1.2	2.18	0.7	0.1274	96.5	17.6	1.6
	115p	0.9	0.374	0.8	0.332	95.6	39.7	2.7
Malida, Midnapur, Bengal	116p	2.1	9.38	5.2	2.32	91.1	40.7	1.6
	117p	0.9	0.174	40.8	7.37	54.8	10.6	3.5
	118p	1.2	0.161	42.4	5.68	53.97	7.22	2.5

\* Represents loss of material in working manipulation

TABLE III—*contd.*

Locality	Soil No.	Heavy fraction		Middle fraction		Light fraction		Error* as per cent of fine sand
		Fine sand (per cent)	Soil (per cent)	Fine sand (per cent)	Soil (per cent)	Fine sand (per cent)	Soil (per cent)	
Mawphlang, Khasi Hills, Assam	119p	0.6	0.072	24.5	2.94	68.9	8.26	6.0
	120p	0.6	0.0678	13.9	1.57	78.5	8.87	7.0
	121p	0.8	0.0936	6.0	0.72	86.9	10.17	6.3
	122p	0.5	0.0475	3.7	0.352	86.7	8.24	9.1
**Garó Hills, Upper Chandmari, Tura	144p	11.9	2.19			87.4	16.1	0.7
	145p	5.8	1.08			93.2	17.3	1.0
	146p	6.6	1.08			92.0	15.1	1.4
	147p	5.80	0.75			93.1	12.0	1.1

\* Represents loss of material in working manipulation

\*\* In the case of these soils the data in the column of light fraction represent both the light and middle fraction

It seems on the whole that the soils generally are fairly rich in their felspar constituents. The proportions of the middle fraction which consist mainly of quartz particles are, however, not so high except in a few cases (e.g. Singbhum, Ranchi and Midnapur).

#### *Estimation of minerals in the heavy fraction of the fine sand*

The separated fine sand fractions were mounted on microscopic slides with Canada balsam by following essentially the procedure described by Milner [1932]. It was found that the nature of the minerals in the middle and in the light fractions of all the soil samples were very nearly the same. The mineralogical composition of these fractions was generally as follows. Mainly quartz and felspar grains and some kaolinized felspars; most of the quartz and felspar grains were found to be stained with limonitic materials; chloritic minerals were also found.

Detailed quantitative mineralogical studies were undertaken with the heavy fractions only.

#### *Determination of different minerals in the heavy fractions*

A square-type graduated micrometer scale was used inside the microscope eye-piece. The microscope tube was adjusted so that the micrometer scale was in the field of view along with the minerals of the microscopic slide and the total number of minerals in the graduated square area were counted. The number of individual minerals in the same area were also determined. By simple process of calculation the percentages by volume of the various minerals in the microscopic slide could be calculated and multiplying these volume percentages by the specific gravity of the minerals, the percentages by weight of the minerals can be obtained. It is, however, necessary to take a large number of counts in order that the calculated percentages by weight of the minerals might be approximately correct. If a large number of counts are taken, it is found that the percentages of the various minerals agree within 10 per cent.

Table IV gives in one place the data on the percentages by weight of various minerals in the heavy fraction of the fine sand along with the nature of the parent materials of the soil types.

TABLE

*Weight of minerals in gm.*

Locality	Soil No.	Depth	Iron oxide**	Zircon	Epidote	Horn-blende	Tourmaline
Hathwara, Manbhumi, Bihar	81p	0-1 ft. 6 in.	87.5	109.0	24.2	35.3	
	82p	1 ft. 6 in.—2 ft. 3 in.	85.5	171.0	19.7	34.8	
	83p	2 ft. 3 in.—3 ft. 6 in.	147.0	232.0	36.0	40.5	5.06
	84p	3 ft. 6 in.—4 ft. 11 in.	79.5	108.0	22.2	15.7	7.84
	85p	Below 4 ft. 11 in.	35.5	36.2	13.4	8.85	1.48
	86p	Below 30 ft.	93.5	1150.0	23.5		
Putida, Singhbhum, Bihar	87p	0-1 ft.			74.0	5.44	
	88p	1 ft.—2 ft. 9 in.	4.34	4.2	3.11		
	89p	2 ft. 9 in.—4 ft.	2.59		0.77		
Ratu, Ranchi, Bihar	90p	0-1 ft.	1050	27.6	30.6	54.0	
	91p	1 ft.—2 ft.	460	5.36	22.3		3.54
	92p	2 ft.—3 ft.	624	8.12	24.0	21.2	
	93p	3 ft.—4 ft.	715	9.24	13.7	24.1	6.02
Baralota, Daltonganj, Bihar	94p	0-1 ft. 11 in.	514			2540.0	36.8
	95p	1 ft. 11 in.—2 ft. 9 in.	302		6.75	675.0	
	96p	2 ft. 9 in.—4 ft.	239			573.0	
	97p	4 ft.—5 ft.	134	217.0		84.6	
Tangi, Cuttack, Orissa	98p	0-1 ft.	30.6		88.5		
	99p	1 ft.—2 ft.	25.1		86.0		
	100p	2 ft.—4 ft.	16.6		39.8		
Dhanmandal, Cuttack, Orissa	101p	0-5 in.	437		360		
	102p	5 in.—4 ft.	333		204		
Kapileswar, Bhubaneswar, Orissa	103p	0-2 ft. 11 in.	1800	47.5	35.1		
	104p	2 ft. 11 in.—4 ft.	2400		44.8		
	105p	Below 30 ft.	675		71.5		
Jhinkartangi, Khurda town, Orissa	106p	0-1 ft.	687	180	66.5		
	107p	1 ft.—2 ft.	935	407	60.2		
	108p	2 ft.—3 ft. 6 in.	1220	494	83.0		
	109p	3 ft. 6 in.—10 ft.	4830	1270	111.0		
	110p	30 ft.—50 ft.	4120	415	307.0		
Lalgah, Midnapore, Bengal	112p	0-4 in.	4000	205	353		578
	113p	4 in.—3 ft. 4 in.	3090	96	71	62.6	282
	114p	3 ft. 4 in.—4 ft.	1470	162	120		211
	115p	7 ft.—8 ft.	273	18.7	24.1		36.6
Malda, Midnapore, Bengal	116p	0-8 in.	6030	463.0	770.0	453	906.0
	117p	Bed soil of Cossaye R.	199.5	8.75	14.5		19.95
	118p	48 ft. below	112.0	8.30	16.9		18.9
Mawphlang, Khasi Hills, Assam	119p	0-6 in.	53.8	2.71	9.35		1.77
	120p	6 in.—1 ft. 3 in.	49.4	4.93	7.38		1.03
	121p	1 ft. 3 in.—2 ft. 1 in.	62.0	4.88	12.30		3.10
	122p	2 ft. 1 in.—4 ft.	31.4	2.38	4.40		3.89
Upper Chandmari, Tura, Garo Hills, Assam	144p	0-3 in.	1930	184.0	77.5		
	145p	3 in.—1 ft. 8 in.	900	104.0	76.7		
	146p	1 ft. 8 in.—2 ft. 8 in.	925	104.0	1.93		
	147p	2 ft. 8 in.—4 ft.	643	10.6			

\*Calculated from the percentages of heavy  
These represent resistant forms of oxides of iron which

## IV

per 10<sup>5</sup> gm. of soil\*

Staurolite	Garnet	Augite	Chlorite	Calcite	Dolomite	Rutile	Biotite	Kyanite	Parent material
43.2	4.5 2.76 6.41 4.95	8.05 7.65 8.88 3.34	3.29 2.02 4.68 1.45 1.37						Granite and gneiss
							75.7		Do.
						72.5 843.0 414.0		126.5 1.7	Dalma traps and Mergui volcanics
									Unclassified crystalline gneiss, etc.
	23.3 7.54 13.7 72.0	41.7 12.2	60.9 13.1 25.0			19.0 6.15 16.7		5.89	Limestone, shales and slates
						118 98 545			Older alluvium and laterite
						1020 435			Laterite mixed with granite and sandstone
		23.8	78.0					44.0 28.1	Gneiss Do.
	6.74				14.0 69.8 329.0 74.0	41.4 69.0			Do.
			45.3	88.5					Do.
274 154 152 15.0			2.82		42.6 89.7 32.8 2.91	44.2 24.9			Older alluvium and laterite Do.
558.0 3.52 5.0		1.61	140.0 1.32		72.2 2.72 1.30	3.83	1.47		Older alluvium and laterite Do. Do.
			1.63 2.01 7.90 0.72		1.69 1.48 1.49	0.83 3.06 2.19 3.29			Shillong series
			3.16						Pab-sandstone

mineral in the soil (Table III)

could not be removed by treatment with oxalic acid

On the basis of the data given in Table IV, the soil profiles studied may be tentatively grouped as follows :—

1. Zircon preponderant . . . (a) Hathwara Farm (Purulia, Bihar)  
(b) Jhinkartangi (Khurda Town, Orissa)  
(c) Upper Chandmari (Tura, Garo Hills, Assam)
2. Hornblende preponderant . . . Baralota (Daltonganj, Bihar)
3. Rutile preponderant . . . (a) Putida (Singhbhum, Bihar)  
(b) Tangi (Cuttack, Orissa)  
(c) Dhanmandal (Cuttack, Orissa)
4. Epidote preponderant . . . (a) Tangi (Cuttack, Orissa)  
(b) Dhanmandal (Cuttack, Orissa)  
(c) Mawphlang (Khasi Hills, Assam)
5. Tourmaline preponderant . . . Midnapore (Bengal).

Mention may be made here of the work of Jeffries [1937], who working with some Pennsylvania soils, has concluded that the percentages of minerals present in the soils can be used as an important aid in soil classification.

#### SUMMARY AND CONCLUSIONS

The present work was undertaken with a view to characterizing some profiles of red and lateritic soils collected from different parts of India by finding out the mineralogical composition of the fine sand fractions of the profile samples, and from the mechanical composition of the soils and the chemical composition of the clay fractions.

In the initial stages of the mineralogical work, the fine sand fractions with which the microscopic determinations were carried out, have not been estimated quantitatively for the percentages of heavy, middle and light fractions. At this stage of the work the heavy and light fractions were separated by shaking with bromoform (sp. gr. 2.9) only, but the separated portions were not weighed. Also the nature of the minerals on the microscopic slides was only qualitatively determined, but they were not counted for determining the percentages of different minerals in the slides.

In the later stage of the microscopical work it was felt desirable to obtain the mineralogical data of the fine sand fractions on a quantitative basis. The fine sand fractions were accordingly separated into three fractions, viz. heavy (sp. gr. 2.9), middle (sp. gr. 2.9-2.65), and light (sp. gr. 2.65) which were weighed separately and their mineralogical compositions determined with the help of a petrological microscope. The percentages of different minerals present in the heavy fractions were also carefully counted with the help of a graduated cross-wire eye-piece micrometer scale.

Common ferro-magnesian rock-forming minerals are represented by one or more species in all the soils examined, one or the other being present in preponderating amount. Hornblende was occasionally present. Chlorite was found in most of the soils studied. Biotite was occasionally found (e.g. Nilgiri hills). These minerals are of resistant nature and they are concentrated in material which has been subjected to prolonged weathering and has accumulated from a variety of sources. Epidote and garnet were frequently found in granular form, whilst tourmaline and especially zircon, though sometimes fragmentary, showed crystalline forms.

Iron minerals like haematite, magnetite and limonite were present in all the soils examined.

With regard to the percentage of the ferro-silicate group of minerals, the soils of basic igneous origin are naturally the richest.

In almost all the cases the proportion of rarer accessory minerals like epidote, zircon, garnet and tourmaline is higher compared with the common rock-forming minerals like biotite and hornblende. The latter minerals are the potential sources of plant food supply and are usually present in the soil samples in small amounts, which explains the comparatively little unproductivity of the soil types.

The general nature and proportions of minerals in the light (sp. gr. less than 2.65) and middle fractions (sp. gr. between 2.9 and 2.65) of all soil samples were found to be very similar. In the light fraction the assemblage of minerals consisted mostly of felspar and grains of quartz often stained with oxides of iron. In the middle fraction, on the other hand, the predominating minerals were quartz grains, mixed with some feldspars, both being stained with oxide of iron.

The outstanding minerals present in the heavy fraction are iron oxide, zircon, tourmaline, staurolite, chlorite, hornblende, epidote and rutile. In a few cases, the existence of minerals like leucoxene, augite, dolomite, calcite and biotite were noticeable.

On the basis of the percentages of the mineralogical constituents in the profile samples, the soil profiles have been tentatively grouped into five classes.

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# DETERMINATION OF ORGANIC PHOSPHORUS IN ALKALI EXTRACTS OF SOILS

BY

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DEAN [1938] outlined a colorimetric method for determining inorganic and organic phosphorus in the alkali extracts of soils. The colour of the extract was removed either by using kieselguhr as an absorbent or by oxidizing it by means of sodium hypobromite at 95°—100° C. The amount of phosphorus determined colorimetrically on the decolourized extract was assumed to be inorganic and the difference of the inorganic phosphorus thus determined and the total phosphorus to the extract determined separately by ashing was taken to represent the organic phosphorus fraction of the extract. The principle underlying this separation is that the organic phosphorus compounds do not liberate any free phosphate ion by the decolourizing treatments. The high temperature at which bromine oxidation was carried out by Dean suggests the possibility of some organic phosphorus being decomposed into inorganic form in which case the separation would become absolutely untrustworthy. Moreover, to get reproducible results it is essentially necessary that the conditions of bromine oxidation should be clearly specified. The work was undertaken with a view to examining the conditions of bromine oxidation, in obtaining a method which would allow the organic colouring matter to be removed without destroying any organic phosphorus compound. For this purpose the alkali extracts of five soils (A. E. A. samples) were decolourized by various treatments as shown below.

## DECOLOURIZING TREATMENTS

(1) Bromine oxidation at boiling temperature, excess bromine being boiled off after acidification (Dean's procedure).

An aliquot of the alkali extract was treated with 5 c.c. of bromine water and heated on a water bath until decolourization was complete. The extract was then acidified to liberate the excess bromine. The bromine was finally boiled off on a water bath until the extract was colourless.

(2) Bromine oxidation at 40°C., excess bromine being boiled off.

The brominated extract was heated on a water bath which was kept at 40°C. The excess bromine was boiled off as in (1).

(3) Bromine oxidation at 40°C., excess bromine being removed at 40°C.

The bromine oxidation was done at 40°C. as in (2). The extract was acidified as before and then heated on a water bath kept at about 40°C. until all the bromine was driven off.

(4) Bromine oxidation at room temperature, excess bromine being removed by aeration.

Bromine water was added to the extract and the extract allowed to stand for some time. It was then acidified as before and a current of air bubbled through it until it was colourless.

(5) Kiesselguhr method (Dean's procedure)—An aliquot of the extract was acidified and heated. The coagulated humus was filtered off and the filtrate shaken with kiesselguhr and again filtered.

(6) Kiesselguhr method (modified)—The humus was coagulated as in (5) but not filtered off before shaking with kiesselguhr.

The inorganic phosphorus was determined in the colourless extract after decolourization had been effected by the above treatments. The total phosphorus of the original alkali extract was estimated by ashing (as shown later) and the organic phosphorus was obtained by difference. The colorimetric method of Deniges [1920] as improved by Truog and Meyer [1929] was used all through. The results showing the organic phosphorus figures only are given in Table I.

TABLE I

*Effect of bromine oxidation at different temperatures on the organic phosphorus in the alkali extract*

(Organic  $P_2O_5$  in mg. per 100 gm. of soil)

Soil	A 1441 Broadbalk head land, heavy loam, acid	A 1442 Woburn, sandy loam, acid	A 1443 Bangor, loam, neutral	A 3328 Carbello, loam, acid	A 2865 King's lynn, sandy loam, alkaline
Treatments					
1. Oxidation and expulsion of bromine at 100°C.	46	24	132	45	23
2. Oxidation at 40°C. and expulsion at 100°C.	48	..	130	..	..
3. Oxidation and expulsion at 40°C.	62	32	156	52	32
4. Oxidation and expulsion at room temperature	64	33	160	56	32
5. Kiesselguhr (Dean's)	64	33	160	56	35
6. Kiesselguhr (modified)	65	33	160	56	33
Total $P_2O_5$ extracted by al- kali	126	74	320	96	72

It will be seen from the table that the temperature of bromine oxidation has a great deal of effect on the organic phosphorus determined. If both

bromination and expulsion of excess bromine are carried out at 100°C. as in (1), the organic phosphorus figures are much lower as compared with the figures obtained by the Kiesselguhr method which shows that at this high temperature the organic phosphorus compounds are considerably, broken down by bromine, to be determined as inorganic phosphorus. It has, of course, been assumed that no such transformation takes place by the treatment with kiesselguhr. When both the operations are carried out at 40°C. as in (3), the results are comparable with those of the Kiesselguhr method. But again if only the expulsion is done at 100°C. as in (2), the figures fall down to the original values as obtained in (1), from which it becomes evident that neither the bromine oxidation nor the expulsion of the excess bromine can be undertaken at 100°C. without affecting the organic phosphorus fraction. Though the results are not affected by the treatments in (3), the removal of excess bromine at 40°C. was found to take more than 2½ hours. Bromination at room temperature and removal of excess bromine by aeration as in (4) compare very favourably with the kiesselguhr and the low temperature oxidation methods. The cold bromination also simplifies the procedure to a great extent, the reaction is completed in a few minutes and the excess bromine can also be removed in a short time. This, therefore, affords a simple and efficient means for the separation of the inorganic and organic phosphorus in the alkali extracts of soils.

As regards the kiesselguhr method it will be seen that the procedure can be a little simplified as in (6) without affecting the results. This method is not only long and tedious but is also incapable of general application. Kiesselguhr can be used as a decolourizing agent only in the case of lightly coloured extracts. Experience showed that even moderately coloured extracts could not be decolourized by it.

Subsequent experience with a large number of determinations on a variety of soils showed that the cold bromination method can be applied successfully to all soils. An occasional highly organic soil may require large amounts of bromine water. While working with some black fen soils giving highly coloured alkali extracts, the author had the opportunity to compare again the original method of Dean with the method proposed by him. Table II contains the results of organic phosphorus determination by the two methods in three successive alkali extracts of two fen soils. Kiesselguhr method was completely ineffective in these cases.

The proposed method gives higher values of organic phosphorus in all the extracts of the two soils. This conclusively shows that the organic phosphorus compounds are considerably decomposed during the process of bromine oxidation at 100°C. as originally proposed by Dean. This breakdown is not, however, proportional to the total organic phosphorus in the extract. It probably depends upon the nature and kind of organic phosphorus compounds and therefore varies from soil to soil. It will be noted that in the second extract of soil A 3560-1 the whole of the organic phosphorus was broken down.

The method finally proposed for the determination of inorganic and organic phosphorus in the alkali extracts of soils may be stated as follows :—

An aliquot of the alkali extract (however obtained) is measured into a 100 c.c. conical flask and treated with a saturated solution of bromine in water,

the volume of bromine required varying from 2 to 15 c.c. according to the colour of the extract. When a large amount of bromine is used, an equivalent amount of alkali should be added if the extract is not sufficiently alkaline already. The brominated extract is allowed to stand for a quarter of an hour and is then acidified with 6N sulphuric acid until bromine is liberated. The flask is then connected to a filter pump and a current of air is bubbled through to remove free bromine. When the extract is colourless it is washed into a measuring flask (50 or 100 c.c.), made up to volume, filtered and the phosphorus determined colorimetrically on an aliquot.

TABLE II

*Comparison of the original method of Dean and the proposed method of determining the organic phosphorus in alkali extracts*  
(Organic  $P_2O_5$  expressed as mg. per 100 gm. of soil)

Soil	Method	1st extract	2nd extract	3rd extract	Total
A 3560-1 Littleport, heavy neutral fen	{ Original method	25.0	0.0	4.0	29.0
	{ Proposed method	54.0	17.0	5.2	76.2
A 3246-47 Peterboro, heavy acid fen	{ Original method	55.0	10.0	5.8	70.8
	{ Proposed method	80.0	17.0	8.7	105.7

The total alkali soluble phosphorus is determined on an aliquot which is evaporated to dryness with 2 c.c. of 10 per cent solution of magnesium nitrate in a porcelain basin and gently ignited. The ignited residue is treated with 1 c.c. of conc. hydrochloric acid, the basin being covered with a watch glass. It is then diluted with water to about 20 c.c. and heated on a sand-bath for 15 minutes. The extract is made to a known volume and phosphorus determined on an aliquot. The organic phosphorus is obtained by difference.

## SUMMARY

1. The colorimetric method for the estimation of inorganic and organic phosphorus in alkali extracts of soils as proposed by Dean has been critically examined.
2. It has been found that the bromine oxidation at 100°C. breaks down a part of the organic phosphorus compounds.
3. On the other hand, the oxidation at room temperature does not decompose any organic phosphorus.
4. A simplified procedure for the separation of organic and inorganic phosphorus in the alkali extracts of soils by bromine has been described.

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# SEEDLING-ADULT COLOUR RELATIONSHIPS AND INHERITANCE IN SORGHUM

BY

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(With Plate IX)

**P**LANT pigments can be classified broadly into two groups, the vegetative and the ornamental. The vegetative pigments are those that are related to the chlorophyll and are therefore indispensable for the normal metabolic activities of the plant. The ornamental pigments have a comparatively less important rôle and though several functions of a physiological and biological nature are attributed to them, they do not seem to be vital to plant life. Sorghum seedlings manifest various types of pigmentation soon after they emerge from the soil and after a few days of growth. Several colour patterns that are associated with chlorophyll exist in sorghum. Under the term 'seedling colours' referred to in this paper are included the colours other than those that are related to chlorophyll. These are confined either to the cell-sap or to the cell-wall and do not exist in the plastid condition like those of the chlorophyll group.

Attempts to classify the sorghum seedlings into pigmentation types have been made in the past. Reed [1930] classified them into two groups, those with red-coloured coleoptiles and those with green coleoptiles. The seedlings with red coleoptiles proved a simple dominant to those with green coleoptiles. Rangaswami Ayyangar [1930, 1932] recorded a simple monogenic segregation between purple and green seedlings. Rangaswami Ayyangar [1934], Rangaswami Ayyangar, Ponnayya and Reddy [1938] refer to the segregation of purple and green coleoptiles. Karper and Conner [1931] made mention of red-stemmed seedlings which behaved as a simple dominant to non-red stemmed seedlings. Fevorow and Havenselman [1934], investigating the hybrids of sorghum and sudan grass, mentioned that the violet colour of the shoot of sudan grass is due to the presence of one dominant factor. Woodworth [1936] reported a 9 : 7 segregation between red-stemmed and green-stemmed seedlings in a cross between Shalalu and Black Spanish ; Rangaswami Ayyangar, Rao and Reddy [1938] have reported a simple segregation of deep purple and purple plumules close on emergence from the soil. The deep purple pigment of the plumules always went with the purple colour of the anthers.

This pigmentation in sorghum seedlings was made use of by plant breeders in several instances. It is an old observation at the Millets Breeding Station,

Coimbatore (south India), that in pure breeding lines of sorghum, odd seedlings possessing a different seedling colour from the type occur very often. These seedlings later on prove to be the resultants of natural crossing. If allowed to grow to maturity they contaminate the inbreeding line still further since sorghum is easily susceptible to cross-pollination and up to as much as 50 per cent of natural crossing is reported in adjacent rows [Ball, 1910]. Hence these seedlings are pulled out in order to prevent further contamination of the inbreeding line. Reed [1930] was able to detect sorghum hybrids at the seedling stage itself with the help of the dominant seedling colour. He selected a parent possessing the recessive seedling colour as mother and pollinated it with a male parent having the dominant seedling colour. The mother parent was not emasculated before pollination. When the seeds of this parent were sown, both the mother type and hybrid seedlings were produced. The hybrid seedlings were readily distinguished from the mother type by the help of the dominant seedling colour which they possessed. Rangaswami Ayyangar [1930] made use of the segregation of purple and green seedlings for demonstrating a simple mendelian segregation graphically by germinating and growing the seedlings on the earhead itself. Vinall, Stephens and Martin [1936] distinguished red and green coleoptiles and made use of this colour for the classification and identification of sorghum varieties of the United States of America.

During the years 1930-36, a large number of sorghum varieties from several important sorghum centres of the world were obtained and grown at the Millets Breeding Station, Coimbatore. Among these, many varieties, especially those from Africa, exhibited certain types of seedling colours which have not so far been recorded. In a previous paper [Rangaswami Ayyangar, 1934] mention has been made of sorghum seedlings with coloured and colourless roots. This aspect was explored and elaborated further; this has led to the knowledge of the existence of several types of root pigmentations. Previous investigators classified sorghum seedlings according to the nature of the shoot pigmentation. The present classification is made based on both the shoot and root pigmentations.

#### CLASSIFICATION OF SORGHUM SEEDLINGS BASED ON PIGMENTATION

##### SHOOT PIGMENTATIONS

The plumules (the seedling shoots) of sorghum seedlings soon after their emergence from the soil are either purple or green. When they are coloured purple they may be either deep purple or purple. The shoot pigmentations are therefore classified into three groups, (1) deep purple, (2) purple, and (3) green. Detailed descriptions of these three groups are given below.

##### *Deep purple shoots*

The coleoptile which is the first to emerge from the soil rapidly develops a deep purple tint (Amaranth purple to Aster purple of plate XII in Ridgway's [1912] *Colour Standards*). The young shoot which pushes out of the coleoptile is pigmented likewise. The first two leaves are coloured deeply like the coleoptile. But in later leaves the pigment gradually lightens up and altogether disappears from the sixth to the eighth seedling leaf upwards. As the shoot grows and when the leaf blades begin to spread out, the pigment disappears

from the blade area and is confined to the region of the leaf-sheath only. In seedlings about a week to ten days old and with about four to five leaves, the pigment is seen only on leaf-sheaths. This pigment on leaf-sheaths is very conspicuous and extends even to the bases of the older leaf-sheaths which are at or near the ground level, so much so that even in seedlings about 40 days old the shoot pigment is easily distinguished.

Besides the manifestation of the pigment on the sheath and the blade areas, the auricular junctions (the specialized tissue that connects the blade with the sheath) also get coloured purple from the third leaf onwards. In sorghum, the junctions of the first two leaves are not well developed and the pigment is not seen in these regions. From the third leaf onwards they get well elaborated [Rangaswami Ayyangar, Rao and Rajabhooshanam, 1938] and the pigment is very clearly noticed. Seedlings over a week to ten days old manifest this pigment well. In seedlings of about 20 days old the exposed portions of the outer margins of the leaf-sheaths also develop purple pigment. The development of the purple pigment in the auricular junctions and the leaf-sheath margins is a characteristic of this class of seedlings and these pigmented organs are unmistakable guides in distinguishing this type of shoot pigmentation from the rest.

#### *Purple shoots*

In this type the manifestation of the pigment is almost parallel to that of the deep purple type, except that the colour is of a much lighter shade of purple (pale Rosolane purple to light Rosolane purple of plate XXVI in Ridgway's [1912] *Colour Standards*). The coleoptile when emerging from the soil is of a pale greenish tint but very soon becomes purple when outside the soil. The plumular leaves as they come out from the coleoptile are also coloured purple. This colour of the plumular leaves is always of a lighter tint than that of the coleoptile. Except the first two seedling leaves the older leaves do not manifest the purple pigment in the blade area. Even in these two seedling leaves the pigment disappears from the leaf-blade, when they have fully emerged from the coleoptile, and is confined to the leaf-sheaths. Similar to the deep purple shoots, in this type also the bases of the leaf-sheaths at or near the ground level are pigmented purple in seedlings of about 40 days old, though in a faint measure. The auricular junctions and the leaf-sheath margins do not develop purple pigment in this type but remain yellowish green. Besides the difference in the depth of the pigment in the young shoots, the non-manifestation of the pigment in leaf-junctions and leaf-sheath margins clearly helps to distinguish this type of shoot pigmentation from the deep purple type.

#### *Green shoots*

In this type the coleoptile and the plumular leaves do not develop purple pigment at any stage of the seedling. The coleoptile when emerging from the soil is either pale yellow or yellowish green. The plumular leaves that push out of the coleoptile are green and remain so thereafter without any trace of purple pigment. The basal region of the older seedling leaves which are at or near the ground level are either yellowish green or green. The auricular junctions and the leaf-sheath margins remain unpigmented. This type

is easily distinguished from the other two pigmented types by the absence of the purple pigment in the coleoptile, plumular leaves, auricular leaf-junctions and leaf-sheath margins.

#### ROOT PIGMENTATIONS

In sorghum as in other mono-cotyledonous plants the primary root soon dies away and a very large number of secondary roots develop, chiefly from the base of the coleoptile. These roots form the mainstay for the growth of the plant. In young seedlings (up to two to three weeks old) the roots are either colourless or occasionally have a light straw colour. But in seedlings over 20 days old they begin to develop their respective colours. They may be either coloured purple or brown. When coloured purple they may be either reddish purple or blackish purple. There are therefore three pigmented groups in roots, viz. (1) Reddish purple, (2) Blackish purple, and (3) Brown. These three groups are described below :—

##### *Reddish purple roots*

These roots at first sight appear brick-red, but on closer examination a faint tint of purple is revealed (Coral-red to Eugenia-red of plate XIII in Ridgway's [1912] *Colour Standards*). The pigment develops first in patches on the older roots in seedlings over 20 days old. These patches gradually begin to coalesce with one another till the entire root looks pigmented. The pigment is not uniformly distributed. On the same root deeper and lighter coloured patches appear. In the early stages of manifestation the pigment is of a lighter tint and gradually deepens later. Generally the older roots get pigmented first, but there is no regularity in colour development on the individual roots. This pigment persists throughout the life of the plant and even after its death in dead tissues.

##### *Blackish purple roots*

In this group the roots are coloured black with a slight tinge of purple (Prune purple to Blackish purple of plate IX in Ridgway's [1912] *Colour Standards*). The development, the manifestation and the persistency of the pigment are similar to those of the reddish purple root pigment. The distinction between the reddish purple and the blackish purple colour is clear enough in freshly washed roots. The difference is more markedly seen when the roots are washed and dried in the sun. The reddish purple roots become brick-red in colour, while the blackish purple roots turn black. On drying, the purple part of the pigment which is common to both these types seems to become unobtrusive, the reddish and the blackish nature of the pigment getting emphasized.

##### *Brown roots*

These roots do not develop purple pigment, but are coloured yellowish brown to brown (Cream buff to Chamois of plate XXX in Ridgway's [1912] *Colour Standards*). The development, the manifestation and the persistency of the pigment are exactly like those of the purple group. This type is easily distinguished from the other two in that the roots do not possess purple colour at any stage of the plant. When the roots are washed free from the soil, the moist roots are coloured yellowish brown. On drying, this yellow colour disappears and the roots look mere brownish.

## DIFFERENT TYPES OF SEEDLING COLOURS

In sorghum seedlings three kinds of shoot pigmentations and three kinds of root pigmentations were described in the previous pages. These shoot and root pigments are two quite different sets of colour manifestations. Any one of the three shoot pigmentations can combine with any one of the three root pigmentations. This combination results in nine different types of seedling colours. These are given below.

Type	Seedlings		Reference to Plate IX
	Shoot colour	Root colour	
1	Deep purple . . .	Reddish purple . . .	Fig. 1. A-D
2	" . . .	Blackish purple . . .	" 4. A-D
3	" . . .	Brown . . .	" 7. A-D
4	Purple . . .	Reddish purple . . .	" 2. A-D
5	" . . .	Blackish purple . . .	" 5. A-D
6	" . . .	Brown . . .	" 8. A-D
7	Green . . .	Reddish purple . . .	" 3. A-D
8	" . . .	Blackish purple . . .	" 6. A-D
9	" . . .	Brown . . .	" 9. A-D

## RELATION OF SEEDLING COLOURS TO ADULT PLANT PIGMENTATIONS

The sorghum plant manifests purple pigmentation in various places and at various stages in its life period, and the inheritance of these pigments have been studied by several investigators. The relationship of the seedling colours with some of these pigmentations are discussed below.

The shoot pigmentation of the seedling were found to have an absolute relationship with the pigment of the leaf-axils—the basal portion of the inside of the leaf-sheath above the region of the nodal band, for brevity called the axil [Rangaswami Ayyangar, Rao and Reddy, 1938]. When the shoots are purple the axils are purple. When the shoots are green the axils are green. In deep purple shoots the axils of the plant are likewise coloured deep purple, and in addition, the nodal bands, the auricular leaf-junctions and the outer margins of the leaf-sheaths extending from the auricular junctions above to the node below, are also coloured purple. The manifestation of the purple pigment in these regions is at its best at the time when the plants are in flower or immediately after flowering. This close association in colour manifestation in both the seedling and the adult has been so constant that not even an exception was met with in the several thousands of pure lines and numerous segregating families studied. Hence it is presumed that the pigmentation in both these places is being brought about by the play of the same set of genes. A close relationship between the deep purple pigmentation of the plumules close on emergence from the soil (the seedling shoots), the axils, the nodal bands, the auricular junctions, the leaf-sheath margins and purple colour of the anthers was reported by Rangaswami Ayyangar, Rao and Reddy [1938]. But in this type of deep purple pigmented shoots the anthers may or may not be coloured purple. When the shoots are deep purple in colour, irrespective

of the fact whether the anthers are purple or not, the axils, the nodal bands the auricular junctions, and the leaf-sheath margins are always coloured purple

The root colours have an absolute relationship with those of the sheath-glume pigmentations reported by Rangaswami Ayyangar [1933]. When the roots are coloured purple, the leaf-sheaths and glumes are also coloured purple. When the roots are brown, the leaf-sheaths and glumes are brown. In the purple group, the reddish purple roots are associated with reddish purple leaf-sheaths and glumes and the blackish purple roots with blackish purple leaf-sheaths and glumes. Beside this concurrent manifestation of the pigment on the root, leaf-sheath and glume, even portions of midrib, leaf-blade and pith that get injured when in active growth develop the corresponding root colour. The pedicelled spikelets, the pulvinor regions of the panicle branches and even the stylar end of the grain, in some cases, develop this root pigmentation.

The nine types of seedling pigmentations and their relationship with the adult plant pigmentations presented are in the tabular statement below :—

No.	Seedling pigmentation			Adult pigmentation	
	Shoot	Root	Axil	Leaf-junctions, leaf-sheath margins and nodal bands	Leaf-sheath and glume
1	Deep purple	Reddish purple	Deep purple	Purple	Reddish purple
2	"	Blackish purple	"	"	Blackish purple
3	"	Brown	"	"	Brown
4	Purple	Reddish purple	Purple	Green	Reddish purple
5	"	Blackish purple	"	"	Blackish purple
6	"	Brown	"	"	Brown
7	Green	Reddish purple	Green	"	Reddish purple
8	"	Blackish purple	"	"	Blackish purple
9	"	Brown	"	"	Brown

#### GENETIC ANALYSIS OF THE SEEDLING COLOURS

##### *Shoot pigmentations*

The inheritance of the shoot pigmentation was pursued in 20 crosses. Of these the inheritance up to the third generation was studied in eight. The results are discussed below :—

A factor **PC** [Rangaswami Ayyangar, 1938] is responsible for the presence of the purple colour in the coleoptile and the young shoot. In its absence (**pc**) the coleoptile and the shoot are green. Purple (**PC**) is a simple dominant to green (**pc**). A factor **PJ** operating in the presence of the purple factor **PC** makes the coleoptile and the shoot deep purple and produces purple pigmentation in the leaf-junctions and leaf-sheath margins in addition. **PJ** has no visible effect in the absence of **PC**. These factor pairs **PCPC** and **PJPJ** result in the following genic constitution of the three groups :—

1. Deep purple **PCPCPJPJ**
  2. Purple **PCPCpj pj**
  3. Green] **pcpcPJPJ**
- or **pcpcpj pj**

The interplay of these two factors results in the usual monohybrid and dihybrid ratios. Segregations for these three shoot pigmentations are presented in the following tables. Tables I—III give simple monogenic segregations and Table IV gives the dihybrid segregation.

TABLE I  
*Pure for pj and segregating for PC*

(Shoots purple to green)

Generation	Family number	Character of selection	Behaviour of progeny	
		Shoot colour	Shoot colour	
			Purple	Green
Parents	A S 2177 .	Purple		
	" 1633 .	Green		
F <sub>1</sub>	" CLIV .	Purple		
F <sub>2</sub>	" 3717 .	.....	202	68
	" 3718 .	.....	375	136
	" 3719 .	.....	152	61
	" 3720 .	.....	340	99
		Total	1069	364
		Expected 3 : 1 ratio	1074.75	358.25
		$\chi^2 = 0.120$	$P > 0.70$	
F <sub>3</sub>	A S 4456 .	Purple . . . . .	186	78
	" 4457 .	" . . . . .	650	237
	" 4460 .	" . . . . .	593	207
	" 4461 .	" . . . . .	361	109
	" 4462 .	" . . . . .	485	115
	" 4463 .	" . . . . .	324	120
	" 4465 .	" . . . . .	63	20
	" 4466 .	" . . . . .	679	216
	" 4470 .	" . . . . .	187	69
	" 4459 .	" . . . . .	Pure	..
	" 4468 .	" . . . . .	"	..
	" 4469 .	" . . . . .	"	..
	" 4465 .	Green . . . . .	..	Pure
	" 4458 .	" . . . . .	..	"
	" 4464 .	" . . . . .	..	"
	" 4467 .	" . . . . .	..	"
		Total	3528	1171
		Expected 3 : 1 ratio	3524.25	1174.75
		$\chi^2 = 0.0159$	$P > 0.80$	

The inheritance of this factor pair **PCPC** was pursued in 40 other families. These gave a total of 6815 seedlings with purple and 2238 seedlings with green shoots in the  $F_2$  generation and 6071 purple and 1958 green in the  $F_3$  generation.

TABLE II  
*Pure for PJ and segregating for PC*  
(Shoots deep purple to green)

Generation	Family No.	Character of selection	Behaviour of progeny	
		Shoot colour	Shoot colour	
			Deep purple	Green
$F_1$ $F_2$	A S 3999	Deep purple		
	" 4847	"	369	128
	" 4848	"	252	83
	" 4849	"	291	107
		Total	912	318
		Expected 3 : 1 ratio	922.5	307.5
		$\chi^2 = 0.4780$	$P > 0.30$	
$F_3$	A S 5725	Deep purple	284	100
	" 5726	"	275	100
	" 5727	"	160	61
	" 5729	"	431	145
	" 5731	"	204	74
	" 5732	"	240	82
	" 5728	"	Pure	82
	" 5730	"	"	82
	" 5733	Green	"	Pure
	" 5734	"	"	"
		Total	1594	562
		Expected 3 : 1 ratio	1617	539
		$\chi^2 = 1.3085$	$P > 0.20$	

The same experience was met with in eight other families and the segregation figures of the  $F_2$  generation are 375 deep purple and 110 green and of the  $F_3$  generation 2547 deep purple and 840 green shoots.

TABLE III  
*Pure for PC and segregating for PJ*  
 (Shoots deep purple to purple)

Generation	Family No.	Character of selection	Behaviour of progeny	
		Shoot colour	Shoot colour	
			Deep purple	Purple
Parents	A S 3447 .	Deep purple		
	„ 60	Purple		
F <sub>1</sub>	„ CCXV	Deep purple . .		
F <sub>2</sub>	„ 4756 .	„ . .	474	167
	„ 4757 .	„ . .	366	127
		Total .	840	294
		Expected 3 : 1 ratio	850.5	283.5
$\chi^2 = 0.5185 \quad P > 0.30$				
F <sub>3</sub>	A S 5511 .	Deep purple . .	235	85
	„ 5512 .	„ . .	210	65
	„ 5513 .	„ . .	204	67
	„ 5514 .	„ . .	Pure	67
		Total .	649	217
		Expected 3 : 1 ratio .	649.5	216.5
$\chi^2 = 0.00154 \quad P > 0.95$				

The above experience was met with in 27 other families. These gave a total of 1386 deep purple and 462 purple in the F<sub>2</sub> and 6376 deep purple and 2029 purple shoots in the F<sub>3</sub> generations.

The dihybrid segregation involving both the factors **PC** and **PJ** was pursued in a family M S 1428 for three generations. M S 1428 is a selection from Tanganyika with purple shoot colour. In it a natural cross A S 3949 with deep purple shoot colour was obtained. When sown, this segregated and a dihybrid segregation involving the three groups, deep purple, purple and green shoots, was obtained. Twenty-three selections were carried forward from this F<sub>2</sub> generation and an F<sub>3</sub> population raised. In the F<sub>3</sub> generation, the dihybrid segregation of the F<sub>2</sub> together with other simple monogenic segregations were obtained. These are given in Table IV :—

TABLE IV  
Segregating for  $PC_t$  and  $PJ$

(Shoots deep purple, purple and green)

Generation	Family No.	Character of selection  Shoot colour	Behaviour of progeny		
			Shoot colour		
			Deep purple	Purple	Green
$F_1$	M S 1428 family				
	A S 3949	Deep purple . . . Expected 9 : 3 : 4 ratio $\chi^2 = 1.6084$	53 47.25	13 15.75 $P > 0.30$	18 21
$F_2$	A S 4812	Deep purple . . .	229	59	91
	" 4813	" . . .	236	81	108
	" 4814	" . . .	215	65	76
	" 4815	" . . .	291	107	119
	" 4820	" . . .	225	67	98
	" 4832	" . . .	63	17	27
		Total . . .	1259	396	519
		Expected 9 : 3 : 4 ratio $\chi^2 = 2.5001$	1229.9	407.6 $P > 0.20$	543.5
	A S 4816	Deep purple . . .	364	121	..
	" 4817	" . . .	138	31	..
	" 4818	" . . .	389	153	..
	" 4819	" . . .	329	81	..
		Total . . .	1220	386	..
		Expected 3 : 1 ratio $\chi^2 = 0.7978$	1204.5	401.5 $P > 0.30$	..
	A S 4834	Deep purple . . .	383	..	125
	" 4835	" . . .	136	..	47
		Total . . .	519	..	172
		Expected 3 : 1 ratio $\chi^2 = 0.00108$	518.25	.. $P > 0.95$	172.75
	A S 4822	Deep purple . . .	Pure	..	..
	" 4823	Purple . . .	..	243	67
	" 4824	" . . .	..	346	96
	" 4825	" . . .	..	212	68
	" 4826	" . . .	..	261	91
	" 4827	" . . .	..	Pure	..
		Total . . .	..	1062	322
		Expected 3 : 1 ratio $\chi^2 = 2.2196$	..	1038 $P > 0.10$	346
	A S 4828	Green . . .	"	"	Pure
	" 4829	" . . .	"	"	"
	" 4830	" . . .	"	"	"
	" 4831	" . . .	"	"	"
	" 4836	" . . .	"	"	"

## GENETIC ANALYSIS OF THE ROOT PIGMENTATIONS

The inheritance of the root pigmentations has been studied in 21 crosses. Segregations up to the third generation were pursued in 11 of these.

The factor **P** [Rangaswami Ayyangar, 1938] is present in the purple group and absent in the brown. Purple is a simple dominant to brown. The purple group is divisible into two sub-groups, one with the factor **Q** [Rangaswami Ayyangar, 1938] which makes the roots reddish purple and the other without it which results in the roots appearing blackish purple. The former is dominant to the latter. These two factors **P** and **Q** result in the following genic constitutions of the three groups.

1. Reddish purple **PPQQ**
  2. Blackish purple **PPqq**
  3. Brown **ppQQ**
- or
- ppqq**

The interplay of these two factors results in the usual monohybrid and dihybrid ratios. Segregations for these three root pigmentations are presented in the following tables. Tables V-VII give simple monogenic segregations for one or the other of the two factors. Table VIII gives the dihybrid segregation.

TABLE V  
*Pure for q and segregating for P*  
(Roots blackish purple to Brown)

Generation	Family No.	Character of selection	Behaviour of progeny	
		Root colour	Root colour	
			Blackish purple	Brown
Parents	A S 4068	Blackish purple		
	" 2380	Brown		
F <sub>1</sub>	" CCLXXVIII	Blackish purple		
F <sub>2</sub>	" 6411	"	355	120
	" 6412	"	210	75
		Total	565	195
		Expected 3 : 1 ratio	570	190
			$\chi^2 = 0.1754$	$P > 0.50$
F <sub>3</sub>	A S 6451	Blackish purple	320	112
	" 6452	"	272	87
	" 6454	"	286	93
	" 6455	"	315	107
	" 6456	"	292	102
	" 6457	"	198	63
	" 6459	"	295	98
	" 6460	"	356	121
	" 6453	"	Pure	..
	" 6458	"	"	..
		Total	2334	783
		Expected 3 : 1 ratio	2337.75	779.25
			$\chi^2 = 0.0240$	$P > 0.80$

The same experience was met with in 18 other families. Three of these gave a total of 410 plants with blackish purple and 145 plants with brown roots in the  $F_2$  generation and the other 15 gave a total of 1305 blackish purple and 384 brown rooted plants in the  $F_3$  generation.

TABLE VI  
*Pure for Q and segregating for P*  
(Roots reddish purple to brown)

Generation	Family No.	Character of selection	Behaviour of progeny	
		Root colour	Root colour	
			Reddish purple	Brown
Parents	A S 3834 .	Reddish purple		
	„ 2487 .	Brown		
$F_1$	„ CCLXXIII	Reddish purple . .		
$F_2$	„ 5272 .	„ . .	287	99
	„ 5273 .	„ . .	156	56
	„ 5274 .	„ . .	325	115
	„ 5275 .	„ . .	205	60
		Total .	973	339
		Expected 3 : 1 ratio	984	328
			$\chi^2 = 0.4918$	$P > 0.30$
$F_3$	A S 6067 .	Reddish purple . .	200	68
	„ 6071 .	„ . .	270	88
	„ 6072 .	„ . .	175	62
	„ 6068 .	„ . .	Pure	..
	„ 6069 .	„ . .	„	..
		Total .	645	218
		Expected 3 : 1 ratio	647.25	215.75
			$\chi^2 = 0.0312$	$P > 0.80$

The same experience was met with in 11 other families. Six families gave 2343 reddish purple and 922 brown in the  $F_2$  and the other five families gave 1316 reddish purple and 483 brown in the  $F_3$  generation.

**TABLE VII**  
*Pure for P and segregating for Q*  
 (Roots reddish purple to blackish purple)

Generation	Family No.	Character of selection	Behaviour of progeny	
		Root colour	Root colour	
			Reddish purple	Blackish purple
Parents	A S 4249 " 29	Reddish purple Blackish purple		
F <sub>1</sub>	" CCLXVI	Reddish purple		
F <sub>2</sub>	" 5429	....	435	138
	" 5430	....	392	126
		Total	827	264
		Expected 3 : 1 ratio	818.25	272.75
		$\chi^2 = 0.374$	$P > 0.50$	
F <sub>3</sub>	A S 6403	Reddish purple	151	45
	" 6306	"	65	25
	" 6307	"	64	23
	" 6305	"	Pure	..
	" 6308	Blackish purple	..	Pure
		Total	280	93
		Expected 3 : 1 ratio	279.75	93.25
		$\chi^2 = 0.0008$	$P > 0.95$	

The same experience was met with in 73 other families. Of these 21 families gave a total of 5537 reddish purple and 1861 blackish purple in the F<sub>2</sub> and the rest 47 families gave a total of 12,489 reddish purple and 4,123 blackish purple in the F<sub>3</sub> generation.

The dihybrid segregation involving the factors **P** and **Q** was pursued in cross A S CCLXXXII each parent contributing one dominant gene. The parents involved are A S 4564, a blackish purple root selection and consequently of the constitution **PPqq**, and A S 2487, a brown root selection of the constitution **ppQQ**. In the F<sub>1</sub> generation both the dominant genes are brought together resulting in the reddish purple roots. In the F<sub>2</sub> generation a dihybrid ratio was obtained. From the F<sub>2</sub> generation a number of selections were carried forward to a third generation and as expected both the dihybrid and monohybrid segregations were obtained. This experience is given in Table VIII,

TABLE VIII

*Segregating for P and Q*

(Roots reddish purple, blackish purple and brown)

Generation	Family No.	Character of selection	Behaviour of progeny		
		Root colour	Root colour		
			Reddish purple	Blackish purple	Brown
Parents	A S 4564 . " 2487	Blackish purple . Brown			
F <sub>1</sub>	„ CCLXXXII	Reddish purple .			
F <sub>2</sub>	„ 6153 .	„ .	268	84	101
	„ 6154 .	„ .	302	98	126
	„ 6155 .	„ .	283	97	125
		Total .	853	279	352
		Expected 9: 3: 4 ratio	834·75	270·25	371
			$\chi^2 = 1·3739$ $P > 0·50$		
F <sub>2</sub>	A S 6462 .	Reddish purple .	165	45	63
	„ 6463 .	„ .	71	26	34
	„ 6464 .	„ .	63	22	31
		Total .	299	93	128
		Expected 9: 3: 4 ratio	292·5	97·5	130
			$\chi^2 = 0·3828$ $P > 0·80$		
	A S 6465 .	Reddish purple .	120	36	..
		Expected 3: 1 ratio	117	39	..
			$\chi^2 = 0·3077$ $P > 0·50$		
	A S 6461 .	Reddish purple .	153	..	49
		Expected 3: 1 ratio	151·5	..	50·5
			$\chi^2 = 0·0594$ $P > 0·80$		
	A S 6466 .	Brown . . . .	..	..	Pure
	„ 6467 .	„ . . . .	..	..	„

The same experience was met with in 28 other families. Sixteen of these gave a total of plants with 3778 reddish purple, 1215 blackish purple and 1663 brown roots in the F<sub>2</sub> generation and the rest 12 families gave a total of 3063 reddish purple, 980 blackish purple and 1354 brown roots in the F<sub>2</sub> generation,

## INTER-RELATIONSHIP OF SHOOT AND ROOT PIGMENTATION GENES

The inheritance of the factor pairs **PCPC** and **PJPJ** determining the shoot pigmentations, and of the factor pairs **PP** and **QQ** determining the root pigmentations have been discussed in the previous pages. The inter-relationship of these four factor pairs will be dealt with in this part. The cross collations and linkages discussed in this part are from artificial crosses designed for the purpose. The linkage relationships are studied both in the coupling and in the repulsion phases and the segregations are pursued up to the third generation. The results are discussed below.

The factor pair **PCPC** is independent of **QQ** and is linked with **P** with a cross-over value of 18.0 per cent. The factor pair **PJPJ** is independent of **QQ** and **PP**. Hence it follows that these four pairs of genes are distributed in three pairs of chromosomes, **PP** and **PCPC** being located in one and the same chromosome. Data relating to the above relationship is presented in Tables IX—XIII.

TABLE IX  
*Crosses with the factor pairs QQ and PC PC*

Generation	Family No.	Behaviour of the progeny				
		Shoot	Purple		Green	
		Root	Reddish purple	Blackish purple	Reddish purple	Blackish purple
Parents	A S 367 . " 3464 .	♀ ..	— —	— —	— —	— ♂
F <sub>1</sub>	" CCVIII	F <sub>1</sub>				
F <sub>2</sub>	" 4391 . " 4393 . " 4394 . " 4396 .		385 365 392 420	128 124 127 134	131 122 142 124	39 38 42 30
	Total .		1562	513	529	149
Expected 9 : 3 : 3 : 1 ratio			1548.56	516.19	516.19	172.06
			$\chi^2 = 3.5447$		$P > 0.30$	
F <sub>3</sub>	A S 5341 " 5346 " 5349 " 5350 " 5352 " 5353 " 5363 " 5364 " 5365 " 5368 " 5370		165 301 256 360 156 115 260 160 143 138 24	52 95 82 111 51 41 82 56 42 43 7	58 101 87 122 56 39 93 52 47 41 7	21 35 30 41 20 12 25 18 18 17 3
	Total .		2078	662	703	240
Expected 9 : 3 : 3 : 1 ratio			2071.69	690.56	690.56	230.19
			$\chi^2 = 1.8425$		$P > 0.50$	

The above experience shows that **PC** is independent of **Q**. Similar dihybrid segregations are obtained in six other families and the total figures are :—

Shoot	Purple		Green	
Root	Reddish purple	Blackish purple	Reddish purple	Blackish purple
Expected	1244	392	385	120
9 : 3 : 3 : 1	1234·31	411·44	411·44	133·81
		$\chi^2 = 4·1187$	$P > 0·20$	

TABLE X

*Crosses with the factor pairs **PJ PJ** and **QQ***

Generation	Family No.	Behaviour of the progeny				
		Shoot	Deep purple		Purple	
		Root	Reddish purple	Blackish purple	Reddish purple	Blackish purple
Parents	A S 3447	—	—	♀	..	—
	" 60	—	—	—	♂	—
F <sub>1</sub>	" CCXV	F <sub>1</sub>				
F <sub>2</sub>	" 4756		356	118	121	46
	" 4757		257	91	95	32
	Total		613	209	216	78
Expected 9 : 3 : 1 ratio			627·8	209·2	209·2	6·98
			$\chi^2 = 1·5402$	$P = 0·50$		
F <sub>2</sub>	A S 4850		170	50	59	18
	" 4961		71	23	20	7
	" 4962		236	98	94	34
	Total		477	171	173	59
Expected 9 : 3 : 3 : 1 ratio			495	165	165	55
			$\chi^2 = 1·5514$	$P > 0·50$		

From Table X it is evident that **PJ** is independent of **Q**. Similar dihybrid segregations were obtained in five other families and the total of these comes to :

Shoot	Deep purple		Purple	
Root	Reddish purple	Blackish purple	Reddish purple	Blackish purple
Expected	460	136	134	45
9 : 3 : 3 : 1	435·94	145·31	145·31	48·44
		$\chi^2 = 3·0482$	$P > 0·30$	

TABLE XI

*Segregations with the factor pairs **PJPI** and **PP***

Family number	Behaviour of the progeny				
	Shoot	Deep purple		Purple	
	Root	Reddish purple	Brown	Reddish purple	Brown
Natural crosses from Family					
A S 1933—					
„ 4774 . . . .		375	120	128	41
„ 4775 . . . .		410	135	138	48
„ 4776 . . . .		270	85	88	25
Total . . . .		1055	340	354	114
Expected 9 : 3 : 3 : 1 ratio		1047·94	349·31	349·31	116·44
		$\chi^2 = 0·4096$		$P > 0·90$	

From Table XI it is seen that **PJPI** and **PP** are independent.

TABLE XII

*Crosses with the factor pairs **PCPC** and **PP***

Generation	Family No.	Behaviour of progeny				
		Shoot	Purple		Green	
		Root	Purple	Brown	Purple	Brown
Parents	A S 2487			♀		
	„ 1745 .				♂	
F <sub>1</sub>	„ CCLXXV		F <sub>1</sub>			
F <sub>2</sub>	„ 5276 .		213	98	90	3
	„ 5277 .		286	125	122	4
	„ 5278 .		550	240	256	8
	Total . . . .		1049	463	468	15
	Calculated 9 : 3 : 3 : 1 ratio		1122·2	374·1	374·1	124·6
			$\chi^2 = 141·8088$		$P < 0·01$	

The high value of  $\chi^2$  shows that the distribution of the four groups does not conform to the normal dihybrid ratio. The  $P$  value is much below 0.01. Such a distribution of the four groups as that obtained above can be explained only on the assumption of a linkage between the factor **PC** for purple shoot colour and the factor **P** for the purple root colour. On this assumption the recombination percentage was worked out and a value of  $18.2 \pm 1.45$  was obtained. On the assumption of linkage with a recombination percentage of 18, the expected distribution of the four groups is as follows :

Shoot  Root	Purple		Green	
	Purple	Brown	Purple	Brown
Numbers obtained . . . .	1049	463	468	15
Calculated (18 per cent crossing over)	1013.66	482.59	482.59	16.16
	$\chi^2 = 2.5515$		$P > 0.30$	

The value of  $\chi^2 = 2.5515$  with  $P$  greater than 0.30 shows that the assumption of linkage with 18 per cent crossing over satisfactorily explains this deviation from the usual simple dihybrid ratio.

From the family A S 5276, two more selections were carried forward and a third generation raised. Both these selections segregated repeating the experience of the  $F_2$  generation. On the assumption of the same linkage as in the  $F_2$  with a recombination value of 18 per cent the distribution of the four groups is as follows :—

Shoot  Root	Purple		Green	
	Purple	Brown	Purple	Brown
A S 6080 . . . . .	192	88	82	3
„ 6081 . . . . .	302	150	148	4
Total . . . . .	494	238	230	7
Calculated (18 per cent crossing over)	492.35	234.40	234.40	7.85
	$\chi^2 = 0.2353$		$P > 0.95$	

The above linkage between the factor pairs **PCPC** and **PP** is in the repulsion phase. The same experience was obtained in the coupling phase also, and the results are given in Table XIII.

**TABLE XIII**  
*Crosses with the factor pairs **PctPct** and **PP***

Generation	Family No.	Behaviour of progeny			
		Shoot	Purple		Green
		Root	Purple	Brown	Purple      Brown
Parents	A S 60 .		♀	—	—
	„ 4174 .	—	—	—	♂
F <sub>1</sub>	„ CCLVIII .		F <sub>1</sub>	—	—
F <sub>2</sub>	„ 5420 .		442	69	65      120
	„ 5421 .		495	70	65      130
	Total .		937	139	130      250
Calculated (18 per cent crossing over)			972.75	119.25	119.25      244.75

$\chi^2 = 5.6662$        $P > 0.10$

From family A S 5420 two more selections were carried forward and a third generation raised. The results are given below :—

Shoot	Purple		Green	
Root	Purple	Brown	Purple	Brown
A S 6296 . . . . .	168	34	35	55
„ 6297 . . . . .	275	35	30	61
Total .	443	69	65	116
Calculated (18 per cent cross- ing over)	462.99	56.76	56.76	116.49

$\chi^2 = 4.7006$        $P > 0.10$

From the above experience it is evident that the factor **PC** for the purple colour of the shoot and the factor **P** for the purple colour of the root are linked with a crossing-over percentage of 18.

#### PIGMENTATION OF THE MESOCOTYL

A feature in which grass seedlings differ from those of other mono-cotyledons in general is the frequent presence of a segment inserted between the cotyledon sucker and the sheath which encloses the plumular bud. This segment is called the mesocotyl [Arber, 1934].

In sorghum, the mesocotyl grows to remarkable lengths and is the chief organ responsible for the emergence of the young shoot from the soil. When the seeds are sown near the ground level, the mesocotyl is short but if they are sown deep, the mesocotyl elongates and thus enables the shoot to emerge out of the soil [Rangaswami Ayyangar and Nambiar, 1934].

Under normal conditions the mesocotyl is always below the soil and it is found that in such cases it always develops the colour of the roots. If the roots are purple, the mesocotyl is purple. If the roots are brown, the mesocotyl is likewise brown. It is noticed that long before the manifestation of the pigment in the roots, the mesocotyl develops the colour and is always an easy guide for determining the root pigmentation. The next step was taken to grow the mesocotyl above the soil in presence of light like the shoot. For this purpose, seeds were sown in trays filled with soil and grown in darkness for two to three days. The seedlings in darkness became etiolated and the mesocotyl region grew out of the soil to a very long length. When examined no trace of the pigment was seen either in the young shoot or in the mesocotyl. The trays were then taken out from darkness and exposed to light. The shoots slowly developed their corresponding pigmentation. It was very interesting to observe that the mesocotyl region also along with the shoot began to develop the shoot pigmentation. If the shoots were deep purple, the mesocotyls developed deep purple colour. If the shoots were purple, the mesocotyls were purple. In green shoots the mesocotyls remained colourless.

Thus it is seen that the mesocotyl which under normal conditions is always below the soil and possesses the root colour when made to grow above the soil in presence of light develops the shoot colour. This differential response of this organ to the development of the pigment under different environmental conditions, behaving sometimes as shoot and sometimes as root, is very interesting and is probably brought about as a result of the change in function of this organ under the different environmental conditions.

#### SUMMARY

The sorghum plant manifests several types of pigmentation in the seedling stage. Both the shoot and the roots develop different types of pigmentations. A classification of the seedlings is made based on both these shoot and root pigmentations.

There are three types of shoot pigmentations, deep purple, purple and green. In the deep purple type, the coleoptile and the young shoot close on emergence from the soil are coloured deep purple, and in the seedling, the auricular leaf-junctions and the outer margins of the leaf-sheaths are coloured purple. In the purple type, the coleoptile and the young shoot are coloured purple, while the auricular junctions and the leaf-sheath margins are not purple pigmented. In the green type, the coleoptile, the young shoot, the auricular junctions and the leaf-sheath margins are green without any purple in them.

There are three types of root pigmentations, reddish purple, blackish purple and brown. These colours manifest when the seedlings are about 20 or more days old. The reddish purple roots are brick-red with a tinge of purple colour in them. When dried they lose the purple tint and become

brick-red. The blackish purple roots are likewise black with a slight tint of purple. On drying the purple tinge disappears and the roots ultimately become black. In the brown type the roots are yellowish brown when fresh and on drying become brown to straw in colour.

Based on these shoot and root pigmentations, sorghum seedlings are classified into nine pigmentation groups :—

These seedling pigmentations have a definite relationship with some of the adult plant pigmentations. When the shoots are deep purple, the leaf-axils are likewise coloured deep purple and the leaf-junctions, the leaf-sheath margins and the nodal bands are coloured purple. When the shoots are purple, the leaf-axils alone are coloured purple and the other regions are green. When the shoots are green, all these regions including the leaf-axils are green without any trace of purple in them. When the roots are coloured purple, the leaf-sheaths and the glumes of the plant are also purple. When the roots are brown, the leaf-sheaths and glumes are likewise brown. The reddish purple roots are associated with reddish purple leaf-sheaths and glumes, and the blackish purple roots with blackish purple leaf-sheaths and glumes. This relation between the seedling and the adult pigmentation is absolute and probably due to the action of the same genes. The mesocotyl develops either the root or the shoot pigmentation according as it is under the soil like the roots or grows above the ground (normally rare) like the shoot.

In inheritance, a factor **PC** is responsible for the purple pigmentation of the young shoot. In its absence (**pc**) the shoot is green. A factor **PJ** acting in presence of **PC** makes the shoot deep purple and colours the leaf-junctions and leaf-sheath margins purple. **PJ** has no visible effect in the absence of **PC**. A factor **P** is present in the purple-coloured roots and absent in the brown. A factor **Q** makes the roots reddish purple ; **q** results in blackish purple roots. The factor **PC** is independent of **Q** and is linked with **P** with a cross-over value of 18 per cent. The factor **P** is independent of **PJ** which again is independent of **Q**.

#### ACKNOWLEDGEMENTS

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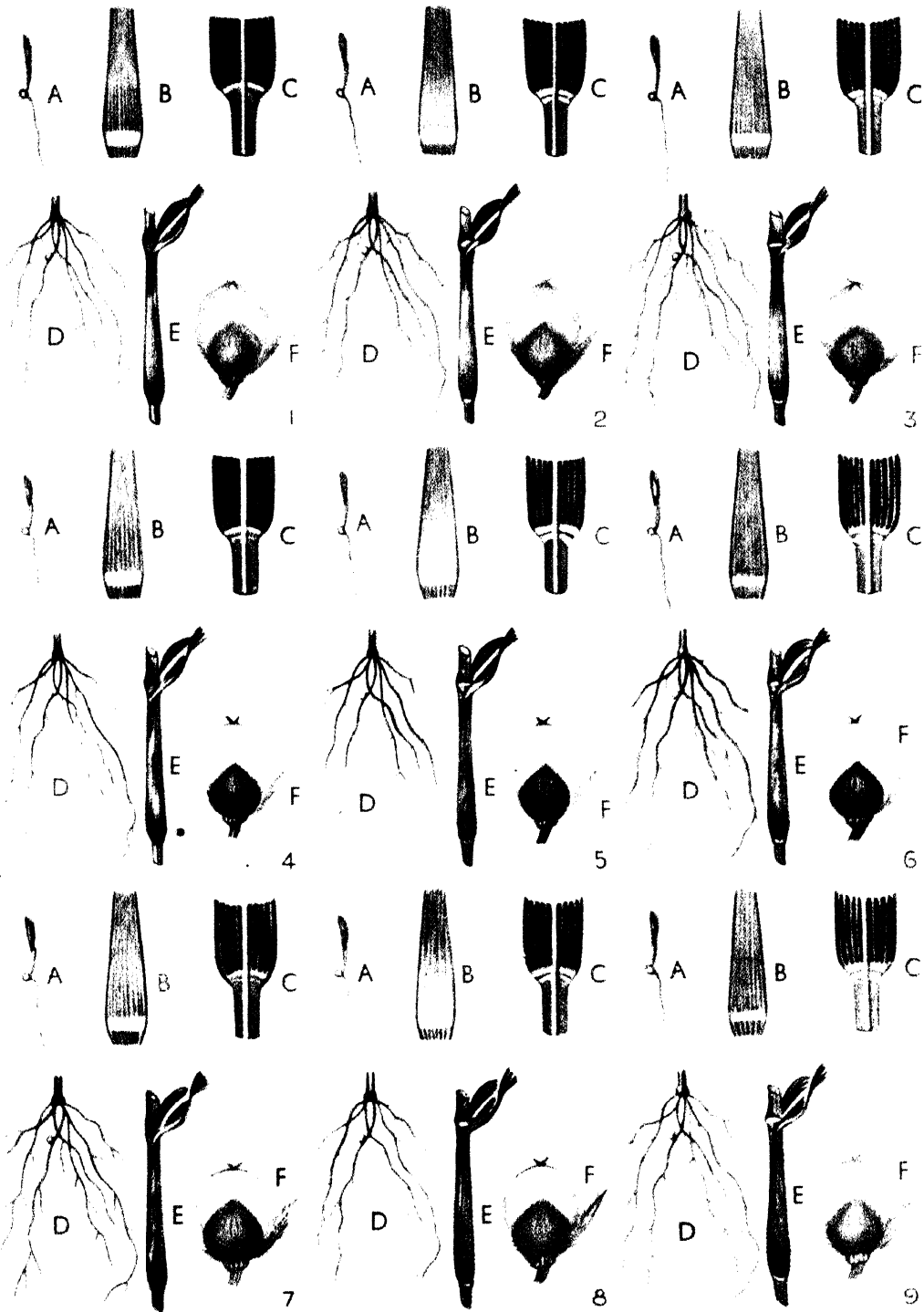
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## EXPLANATION OF PLATE IX

The plate is divided into nine sections, numbered 1 to 9. In these nine sections are shown the nine pigmented types of sorghum seedlings and the related pigmented parts of the adult.

Section No.	Seedlings	
	Shoot	Roots
1	Deep purple . . . . .	Reddish purple
2	Purple . . . . .	" "
3	Green . . . . .	" "
4	Deep purple . . . . .	Blackish purple
5	Purple . . . . .	" "
6	Green . . . . .	" "
7	Deep purple . . . . .	Brown
8	Purple . . . . .	"
9	Green . . . . .	"

In each section, A represents a seedling five days old ; B leaf-axil ; C leaf-junctions ; D roots of a seedling 20 days old ; E internode with the leaf-sheath at maturity showing the nodal bands ; and F mature glumes enclosing a well-developed grain.



{ For explanation see opposite page



In the deep purple shoot type (Nos. 1, 4 and 7), the coleoptile and the plumule of the seedling A, the leaf-axil B, the leaf-junctions C and the nodal bands in E, are pigmented deep purple. In the purple shoot type (Nos. 2, 5 and 8), the coleoptile is light purple and the plumular leaves are merely tinged purple; the leaf-axil is light purple, while the leaf-junctions and the nodal bands are green. In the green shoot type (Nos. 3, 6 and 9) the coleoptile, the plumular leaves, the leaf-axil, the leaf-junctions and nodal bands are green without any trace of purple in any one of these parts.

In the reddish purple root type (Nos. 1, 2 and 3), the seedling roots D, the mature leaf-sheath E and the glumes F, are pigmented reddish purple. In the blackish purple type (Nos. 4, 5 and 6), all these parts are pigmented blackish purple, and in the brown type (Nos. 7, 8 and 9), these are brown.

# EFFECT OF COTTON SEED DISINFECTION ON YIELD

BY

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DAMPING-OFF and seedling-blight diseases of cotton are caused in these provinces by *Pythium* sp., *Macrophomina phaseoli* Ashby, *Rhizoctonia solani* Kuhn., *Rhizoctonia* sp., *Sclerotium Rolfsii* Sacc. and *Colletotrichum indicum* Dast. These fungi often cause poor germination and considerable loss of seedlings. Trials were carried out for the control of these diseases by treating the seed. During the trials it was found that the incidence of these diseases is not annual and not always of the same intensity. The predisposing factors are excessive moisture caused by heavy rainfall and water-logging during the critical period, the first three or four weeks after germination, when the seedlings are most susceptible to infection by these fungi, and, in the case of the anthracnose disease (*Colletotrichum indicum*) of seedlings, the use of infected seed. For these reasons the value of seed treatment for the control of these diseases is not well established except in the case of the seed-borne anthracnose disease [Dastur, 1934]. However, there is evidence that the mortality of seedlings raised from disinfected seeds is less than that of seedlings from untreated seeds in years when there is an epidemic of these diseases. But these trials have shown the value of seed disinfection in increasing the yield.

In Nagpur, these trials have been carried out since 1936 and for the last two years these treatments have been tried on some of the district farms as well. The seed treatments tried at Nagpur were dusting with Agrosan G, Hortosan B, Abavit B, Ceresan, finely powdered copper carbonate and sulphur, and delinting with sulphuric acid. The four proprietary fungicides were used at the rate of one ounce per 28 lb. of seed; the two chemicals were used at the rate of two ounces per 28 lb. of seed; for delinting the seed with sulphuric acid one part by volume of the acid was used for 20 parts by volume of the seed.

It is a general practice amongst cultivators of the province to wet the cotton seed with a cowdung solution so that, on drying, the fuzz is set and the seed passes readily through the drill. The fungicide in the powder form was well mixed with the cowdung solution before it was applied to the seed.

Delinting with sulphuric acid was carried out as described in a previous publication [Dastur, 1934].

In 1936 each treatment was tried on 1/40 acre plot and replicated three times. Each block consisted of these seven treatments and one control. The three blocks were placed contiguously. The following year these trials were repeated; each treatment and the control were tried on 1/17 acre plots and replicated three times. In 1938, these trials were again carried out with the same number of replications but each plot was 1/20 acre.

It was observed that the delinted seeds germinated earlier by a day or two than the other seeds. The plants in the control plots were at first smaller than those in plots sown with treated seed. This difference in growth was noticeable only up to about two months after sowing ; but when the flowering stage was reached, there was no visible difference in the size of the plants.

The yield of *kapas* (seed cotton) from the plots sown with treated seed was higher than that from the control plots during these three years.

As the treated seed in the trials had given a higher yield than the untreated seed, it was considered desirable to increase the number of replications and to test only those fungicides which are readily procured in India and which can be easily used by the cultivator. Ceresan is not marketed in India ; delinting seed with sulphuric acid cannot be done by the ordinary cultivator ; therefore, these two treatments were not tried again. In 1939 and 1940, each plot was 1/20 acre and the replications were six ; the treated and untreated seeds were sown in randomized plots. The percentage of increase in yield from the five years' trials are given below :—

Treatment	1936-37	1937-38	1938-39	1939-40	1940-41
Agrosan G . . . .	14.4	27.0	38.3	19.0	1.7
Hortosan B . . . .	9.1	20.6	44.6	21.1	21.2
Abavit B . . . .	8.0	13.9	40.3	17.5	5.9
Ceresan . . . .	16.3	25.9	25.9	..	..
Copper carbonate . .	25.3	24.9	38.9	8.2	12.7
Sulphur . . . .	15.2	29.9	33.7	19.5	12.4
Delinted seed . . . .	8.7	9.9	10.4	..	..

In 1940, the monsoon was unseasonable and, when it broke, the rains were abnormally heavy and continuous ; the result was that all the six blocks could not be sown on the same day ; a block and a half were sown on 30 June ; further sowing could not be done till 8 July when only 3½ blocks could be sown ; the remaining sixth block could not be sown on account of rain. No further sowing was possible till 25 July. The fields got flooded and some of the plots sown on 8 July were under water for many days ; either seeds were washed away or seedlings were killed. For these reasons the yield from only a few plots could be considered, those that were sown in the end of June and some of those that were sown on 8 July. Last year's results cannot be therefore compared with those of the previous four years,

In 1939 and 1940, three of these fungicides were tried out on the Akola Farm. Each plot was  $\frac{1}{4}$  acre and replicated four times. The plots were randomized. The percentage of increase in yield is given below :—

Treatment	1939-40	1940-41
Agrosan G . . . . .	11.3	12.5
Copper carbonate . . . . .	11.8	4.7
Sulphur . . . . .	10.6	1.9

At the Government Farm, Khandwa, in 1939 seed treated with Agrosan G and copper carbonate were sown on 0.3 acre plots with six replications. The control plot was also replicated six times. In 1940, the size of each of these three plots was  $\frac{1}{20}$  acre; each plot was replicated six times. These trials were carried out in randomized plots. The percentage of increase in yield is given below :—

Treatment	1939-40	1940-41
Agrosan G . . . . .	26.5	10.4
Copper carbonate . . . . .	12.4	6.8

On a demonstration plot at Amraoti, Agrosan G and sulphur treatments were tried in 1939 and 1940. The 1940 trials were vitiated on account of the unseasonal and abnormal rainfall, and therefore the results of the 1940 trials cannot be considered. In 1939, seeds treated with Agrosan G and with sulphur were sown on two-acre plots each and the untreated seed was sown on a one-acre plot. The percentage of increase of yield from the plots sown with seed treated with Agrosan G and sulphur was 16.2 and 11.5 respectively.

On the Government Farm at Ellichpur in 1939, a field of 10 acres was divided into four equal plots of  $2\frac{1}{4}$  acres each; one plot was sown with seed treated with Agrosan G, another plot with copper carbonate-treated seed and the remaining two plots were sown with untreated seed and alternated with the plots sown with the treated seed. The yield from the plot sown with seed treated with Agrosan G was 14.7 per cent higher than that from the control plots, but the yield from the plot sown with seed treated with copper carbonate was less by 19.4 per cent,

The following year these two fungicides were tried again on this Farm in two fields separated from each other. In one field each plot was 1/3 acre and replicated five times. On the second field these trials were repeated six times in randomized plots of 1/20 acre each. The percentage of increase in yield from these two plots is given below :—

Treatment	Field I	Field II
Agrosan G . . . . .	12·6	7·6
Copper carbonate . . . . .	7·6	5·3

It is not possible to give a precise explanation for the lower yield obtained from the plot sown with copper carbonate-treated seed in 1939. But since the following year on two fields on the same Farm the yield was higher than from the control plots, it is permissible to assume that the lower yield in 1939 could not be due to the treatment.

In 1940, some of these treatments were tried at Borgaon and Yeotmal Farms. Each of the plots sown with treated and untreated seed was 1/20 acre and replicated six times. The percentages of increase in yield are given below :—

Treatment	Borgaon Farm	Yeotmal Farm
Agrosan G . . . . .	2·0	31·2
Copper carbonate . . . . .	6·8	19·0
Sulphur . . . . .	9·6	32·5

The results of these experiments, which have been carried out for many years and on various Government Farms, show that chemical treatment of cotton seed increases the yield of *kapas* (seed cotton); the percentage of increase is variable, but is sufficient to justify the use of treated seed; seed treatment is inexpensive and does not need any extra labour or skill or care. The treatment with fungicides not only ensures increase in yield but also is capable of destroying fungus spores, e.g. spores of the anthracnose fungus; this disinfection of the seed would naturally ensure better germination and reduction in loss from disease, especially when conditions are unfavourable for quick germination.

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# STEM-BROWN DISEASE OF APPLE IN KUMAUN

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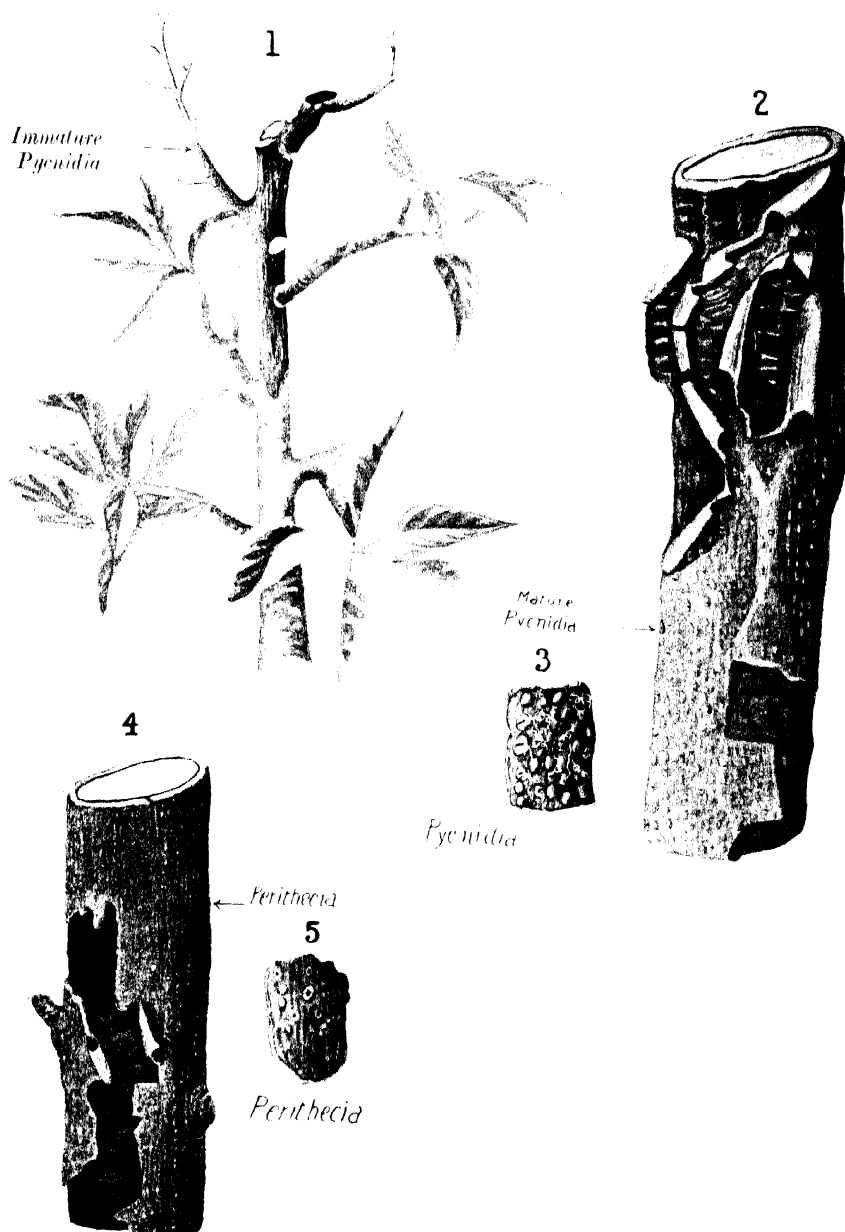
(With Plates X-XIV)

A SERIOUS disease of apple stem caused by *Botryosphaeria ribis* Gross and Dugg. was recorded for the first time in India in August 1934 in the Government Orchard, Chaubattia (Kumaun), and a short note regarding the disease was given in the Annual Report of the Hill Fruit Research Scheme, Chaubattia for the year 1934-35. The specimen was sent to Mr Ashby, the Director of the Imperial Mycological Institute, Kew, who identified it as *Botryosphaeria ribis* Gross. and Dugg. and he enquired whether the fungus in culture formed a red colour on starch or not, which the parasitic strains of *B. ribis* were known to produce. Since it produced this colour on starch in cultures, the fungus proved to be identical with *B. ribis*. This fungus has had a great variety of names applied to it in its different stages. The ascogoneous stage is best known in Europe under the name of *Botryosphaeria Berengerians* de Not. In America it has been frequently called *Botryosphaeria fuliginosa* (M+N) E & E. The occurrence in the Hudson Valley of a destructive blight or wilt of currants was first described by Fairchild [1891] and ascribed to a 'sterile' fungus. The importance of *Botryosphaeria ribis* as a pathogene of currant and gooseberry was demonstrated by Grossenbacher and Duggar [1911]. They described the perfect stage as *B. ribis*. The imperfect stage common on many hosts is a *Dothiorella*. Putterill [1919] described a canker of apple trees in South Africa, which was caused by a fungus closely resembling *Botryosphaeria ribis*. He called it *Botryosphaeria mali*, the difference between it and *B. ribis* being in the width of asci and in the size of the stroma. Recently Stevens and Jenkins [1924] have shown that the fungus occurs on horse chestnuts and is parasitic on roses causing canker on the stem and sometimes killing the whole canes. An exhaustive history of the fungus and its host range is given by Smith [1934] who found the fungus to have a wide host range, including at least 34 genera and 20 families of plants, one of which is the apple. Birmingham [1924] recorded *Dothiorella mali* as causing a stem disease of apples in Australia. *Botryosphaeria ribis*, thought to be the perfect stage of *D. mali* was found to cause rotting of apples by Fenner [1925]. Hopkins and Bacon [1938] recorded this disease on apple stem in Southern Rhodesia.

## SYMPTOMS OF THE DISEASE

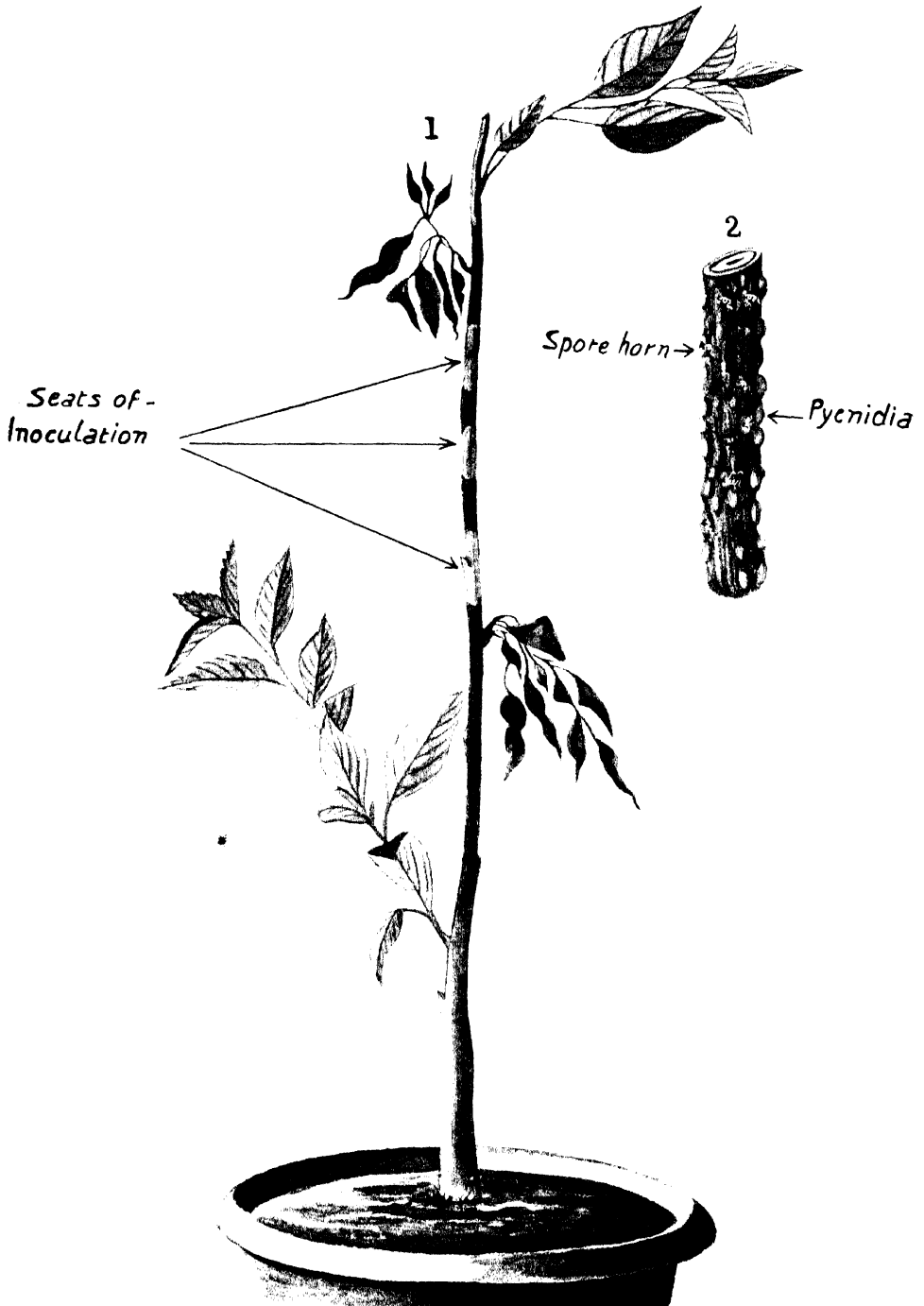
The disease starts from the pruned surface of twigs and stems and proceeds downwards causing a type of die-back (Plate X, fig. 1). The upper limbs of the apple trees are more liable to the attack. It causes a loosening

STEM-BROWN CAUSED BY *Botryosphaeria ribis*



1. Stem-brown disease on a young shoot ( $\times \frac{1}{3}$ )
2. Stem-brown disease on old shoot showing pycnidia ( $\times 1\frac{1}{3}$ )
3. Pycnidia enlarged ( $\times 2\frac{2}{3}$ )
4. Stem-brown disease on an old shoot showing perithecia ( $\times 1\frac{1}{3}$ )
5. Stem-brown disease on perithecia enlarged ( $\times 2\frac{2}{3}$ )

MYCNIDIAL STAGE OF *Botryosphaeria ribis*



1. One-year-old plant of Esopus Spitzenberg artificially inoculated at three places ( $\times 4$ )
2. An enlarged portion of the infected stem showing pycnidia 'B' type with spore horns ( $\times 13$ )

of the bark which becomes papery and brown and rolls outwards (Plate X, fig. 2). On removing the bark, the wood is found to be stained dark brown in colour and fissured both horizontally and longitudinally (Plate X, fig. 2). A combined attack of the fungus and *Coniothecium chomatosporum* Corda, which caused another similar disease, is often found to be present in the cankered areas. The disease is noticed usually by the fourth week of April and is in the most virulent form by the middle of May. In old herbarium specimens pycnidia and perithecia are met with. Perithecia are rarely found in nature.

The mycelium of the fungus is found right up to the wood and is dark brown in colour, closely septate tending to form chlamydospores. The fruiting bodies, especially pycnidia of simple type, are formed just outside the cork-forming layer which is depressed by their growth. The primary cortex is raised to form marked protuberances. Compound pycnidia form small globular sclerotial bodies between the phellogen and outer cortical parenchyma of shoots and stems.

### MORPHOLOGY OF THE FUNGUS

#### PYCNIDIA

Two types of pycnidia are met with in the old diseased specimens of the apple stem. For convenience these two types will be referred to in this paper as pycnidia-A and pycnidia-B.

#### *Pycnidia-A*

They are very minute structures dotted all over the stem. Their conceptacles are sclerotoid, immersed, crowded with distinct ostioles in the mature stage and measure 0.25-0.5 mm. There is a small stroma which is sometimes confluent. The central core of the conceptacle remains a solid pseudoparenchyma of thick-walled, soft cells, and around this in the hyaline part of the stroma irregular cavities are formed (Plate XII, figs 4 and 5). These cavities become irregularly lined with pycnosporo-mother-cells which form within them bacillar, allantoid spores measuring  $9-12 \times \pm 1\mu$  which are hyaline and apparently continuous. The spores seem to be set free by the dissolution of the spore-mother-cells and are thus endogenous.

The pycnosporo-sporangia are regarded as spermatia, and all attempts to germinate them have been unsuccessful. There are some points of resemblance of these to the filiform bodies figured by Klebahn [1933] for *Sclerophoma strobili*. This pycnidial stage of the fungus may well be termed the sclerophoma stage. These pycnidia-A are formed in the vicinity of stromata destined to give rise to perithecia. Spear [1910] also described a similar type of small, hyaline, cylindrical pycnosporo-sporangia measuring  $2-3\mu \times 1\mu$  which he found on the host only and not in culture.

Similar structures were also described by Grossenbacher and Duggar [1911], but they considered that these did not belong to the life-cycle of *Botryosphaeria ribis*. The spores failed to germinate. Tulasne and Tulasne [1863] described and figured a similar stage for their *Dothiorella melanops*, now usually called *B. melanops* (Tul.).

### *Pycnidia-B*

In nature pycnidia-B are found in greater abundance than pycnidia-A. They are minute, osteolate bodies (Plate X, fig. 3) and at times give out spore horns light pink in colour. They are either simple or compound stylosporic form (*Dothiorella*) and are borne in the same or similar stroma as the perithecia.

Pycnidia-B are either single or in groups of two to six, globose, black, erumpent, ostiolate having a thick wall (Plate XII, fig. 1). They measure  $126\text{--}394 \times 114\text{--}8\text{--}210\mu$ ; the average size being  $220\cdot7 \times 166\cdot3\mu$ . The conidiophores are small, unbranched, hyaline, bearing pycnosporos at the tip. The pycnosporos are fusoid to oblong, elliptical, hyaline to sub-hyaline, unicellular and measure  $9\cdot1\text{--}25\cdot6 \times 5\cdot6\text{--}7\cdot7\mu$ ; average  $17\cdot52 \times 6\cdot8\mu$  (Plate XII, figs. 3a-3o). These pycnosporos germinate readily in tap water within 8-12 hours and send out germ tubes from either end (Plate XII, figs. 6a-6f).

### PERITHECIA

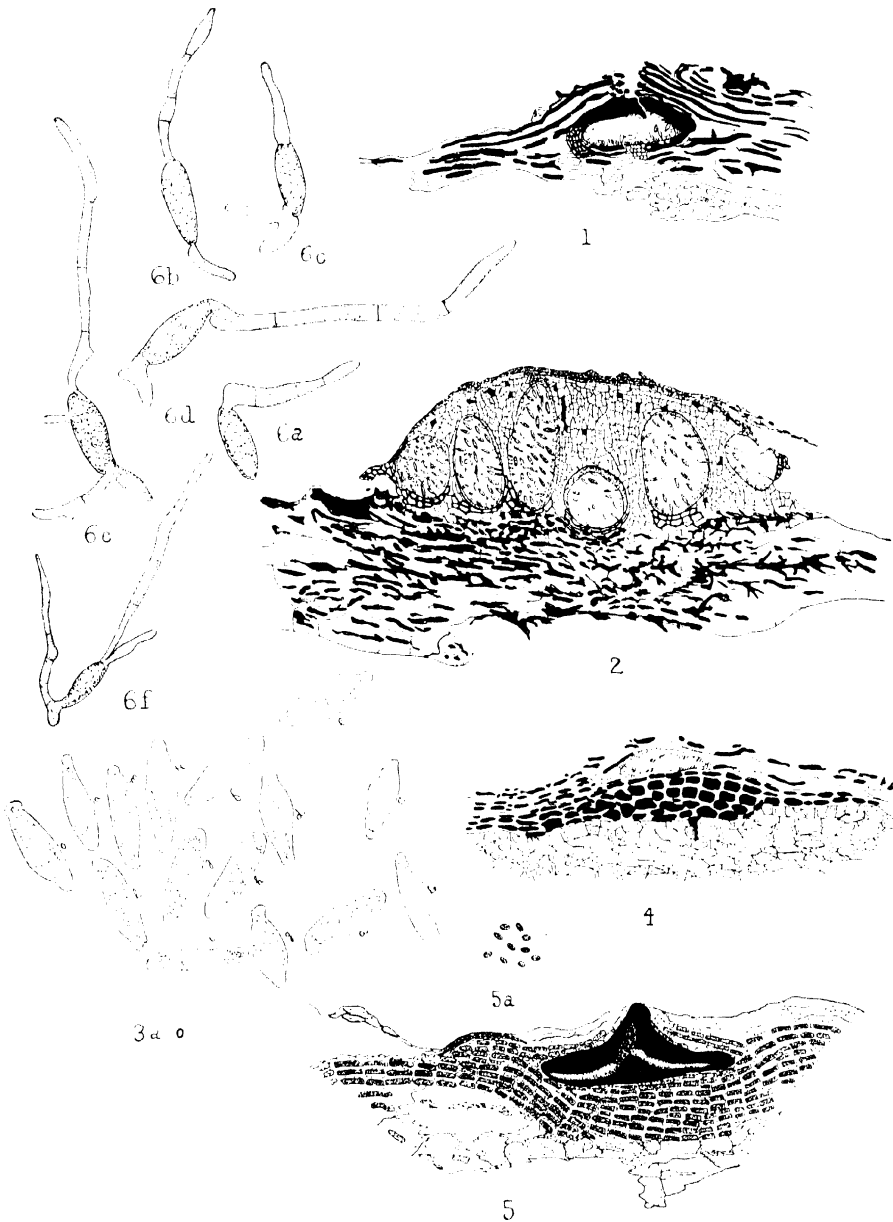
The perithecia are not so commonly met with in nature as the pycnidia. They are round, top-shaped bodies with papillate ostioles (Plate X, figs. 4 and 5). They either occur singly or in groups of two to eight (Plate XIII, figs. 1 and 2). The stroma are black, more or less pulvinate, and measure from 0.5 to 5 mm. in diameter. The perithecia are sometimes interspersed among pycnidia. The perithecia measure  $140\cdot0\text{--}252 \times 127\cdot4\text{--}280\mu$ ; average  $190\cdot7 \times 192\cdot0\mu$ . The asci are clavate, eight spored, hyaline and measure  $36\cdot4\text{--}112\cdot0 \times 14\cdot0\text{--}18\cdot9\mu$ ; average  $77\cdot5 \times 15\cdot4\mu$  (Plate XIII, figs. 3a, 4 and 5a-5d). The ascospores are biserate in arrangement, unicellular, hyaline, fusoid, elliptical to ovoid (Plate XIII, figs. 6 and 7a-7d) and measure  $11\cdot9\text{--}28\cdot00 \times 8\cdot4\text{--}12\cdot6\mu$ ; average  $20\cdot2 \times 10\cdot7\mu$ . The paraphyses are present and are filiform. The ascospores germinate in tap water at room temperature in 10-14 hours throwing out germ tubes from either end. (Plate XIII, figs. 8a-8d). Affected pieces of apple twigs bearing pycnidial structures were kept immersed in the snow for one month and, when examined in March, were found to contain mature and immature perithecia. What part the perithecia play in the spread of the disease is difficult to say, but at any rate there is clear indication that old affected twigs of apple bearing pycnidia if buried in the winter snow will undoubtedly be a potential source for the spread of the disease in the following spring.

### CULTURAL CHARACTERS

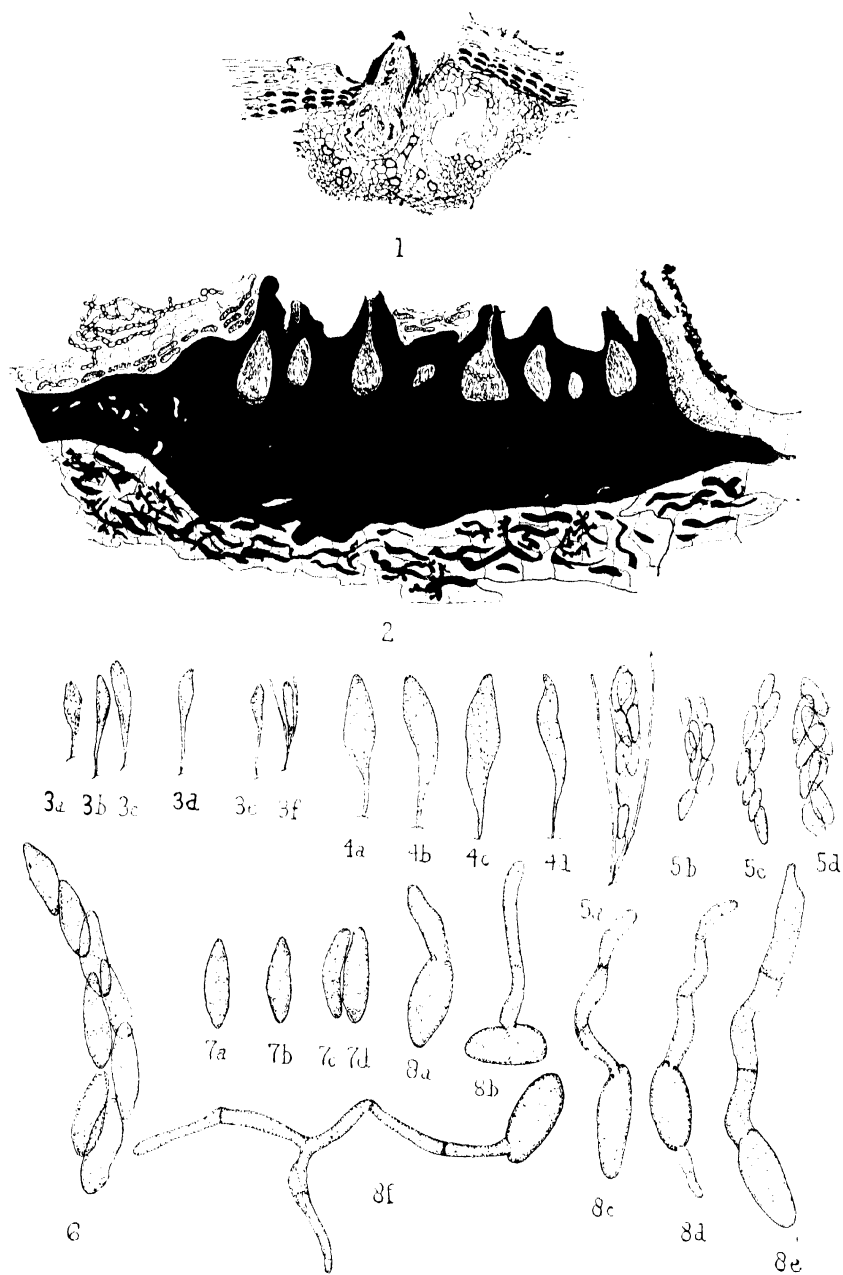
The cultural studies of the three isolates of *Botryosphaeria ribis*, no. XXI (from single pycnosporos), St-Br (from single ascospore) and 123 (from Holland) (Plate XIV, figs. 1 and 2) were carried out in detail on three media, Czapek's agar, potato-dextrose agar and Brown's starch synthetic agar.

#### *Linear measurements of growth*

All the cultures were grown in petri dishes of equal size and the amount of medium in each was 10 c.c. Each treatment was replicated six times and the four measurements of each of the colonies were taken. All the petri dishes were kept at a constant temperature of 30°C. in an incubator.



1. Transverse section of bark of apple stem showing single pycnidia 'B' type (*Dothiorella*) ( $\times 107$ ); 2. Transverse section  $8\mu$  thick of bark of apple stems showing pycnidia 'B' of the compound stylosporid form ( $\times 107$ ); 3a-30. Pycnosporos from pycnidia 'B' type ( $\times 1,066$ ); 4. Transverse section of bark of apple stem showing pycnidia 'A' type (*Sclerophoma* type) ( $\times 107$ ); 5. Transverse section  $8\mu$  thick of bark of apple stem showing pycnidia 'A' type (*Sclerophoma* type) ( $\times 107$ ); 5a. Pycnosporos from Pycnidia 'A' type ( $\times 1,066$ ); 6a-6f. Pycnosporos 'B' germinating ( $\times 1,066$ )



1. Transverse section of bark of apple stem showing perithecia ( $\times 107$ );  
 2. Transverse section of bark of apple stem showing group of perithecia ( $\times 107$ );  
 3a-3f. Immature asci ( $\times 480$ ); 4a-4d. Immature asci ( $\times 1,066$ ); 5a-5d. Mature  
 asci with ascospores ( $\times 480$ ); 6. Eight ascospores ( $\times 1,066$ ); 7a-7d. Ascospores  
 ( $\times 1,066$ ); 8a-8f. Ascospores germinating ( $\times 1,066$ )

It was noted that the isolate XXI grows best on Brown's starch synthetic agar, the isolate St-Br, on Czapek's agar and the isolate 123 on potato-dextrose agar. Thus all the three isolates differ from each other as far as the linear rate of growth of the cultures is concerned.

#### *Macroscopic and microscopic characters*

Macroscopic and microscopic characters were also noted for the three isolates. All the isolates differ in macroscopic and microscopic characters.

No pycnidia or perithecia were formed by any of these isolates in dark at a temperature of 30°C., but in light, at room temperature, the strain XXI formed pycnidia of 'B' type on potato-dextrose agar only, while the isolate of St-Br produced them in all the three media. Photographs of the three isolates growing on potato-dextrose agar are given in Plate XIV, figs. 1a-1c and the paired cultures in petri dishes are shown in Plate XIV, figs. 3a and 3b.

#### CULTURAL STUDIES OF THE SALTANTS

A saltant appeared in two culture flasks of the local strain No. XXI (Plate XIV, figs. 2a and 2b). They were given the numbers XXIa and XXI aI. They were isolated and grown at 30°C. on Brown's starch synthetic agar, potato-dextrose agar and cornmeal agar for comparison of cultural character with the parent strain No. XXI and also with the strain St-Br. All the cultures were kept in the dark. The principal difference was found to be in the rate of their linear growth which was faster than the parent, but slower than the isolate St-Br. On cornmeal agar and potato-dextrose agar both the saltants produced more broad and distinct zones than the parent isolates. None of them showed zonation on Brown's medium.

#### INOCULATION EXPERIMENTS

Two isolates of the fungus were taken. One of the strains numbered XXI was isolated from diseased apple twigs bearing pycnidia-B in bacto-agar from the growth of a single germinated pycnospore. The other strain numbered St-Br was isolated from a single ascospore.

The two isolates differ from each other in cultural characters. They both produce the dothiorella stage but no perithecia. A third foreign isolate of *Botryosphaeria ribis* received from Central Bureau Schimmelcultures Baarn (Holland) which was numbered 123 did not produce even pycnidia but remained altogether sterile. All the three strains caused chromogenesis of starch in light.

For establishing the pathogenic characters of the fungus the following experiments were carried out :—

#### *Entry of the fungus through injured surfaces*

In April 1937, 12 plants of *Esopus Spitzenberg* grafted on seedling stocks (age of the graft was one year) were selected. In all cases the upper portion of the stem was injured aseptically at three places, and the inoculum was placed over the injury which was then covered over with wet absorbant cotton and finally with cellophane bags (Plate XI, figs. 1 and 2). On the

lower portions of the stem the inocula were placed at three uninjured places and similarly covered. Thus we have the following arrangements :—

1. Plants A, B and C inoculated with culture No. XXI
2. Plants D, E and F inoculated with culture No. St-Br
3. Plants G, H and I inoculated with culture No. 123
4. Plants J, K and L uninoculated controls

All the inoculated and control plants were kept inside a glass cage the air of which was kept constantly humid. After about a month only the injured spots were observed to have taken the infection, while the uninjured ones and the controls remained unchanged.

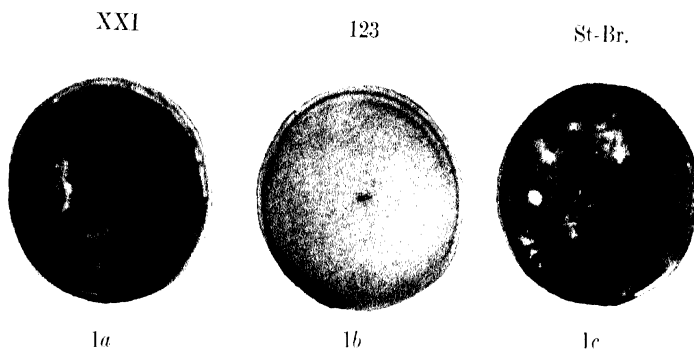
The inoculated portion at first becomes depressed and becomes light brown in colour. The area of the stem below and above the inoculated portion also turns light brown, its bark becoming thin and papery. In advanced stages the whole of the infected stem turns dark brown and gets studded with innumerable pycnidia which when mature give out light pink spore horns (Plate XI, figs. 1 and 2). They were all B type of pycnidia, belonging to dothiorella stage of *Botryosphaeria ribis*. Pycnidia-A did not appear in any of the infected portions. Perithecia were observed the following year (in December 1938). The progress of infection in all cases was very slow.

#### CROSS-INOCULATION EXPERIMENTS

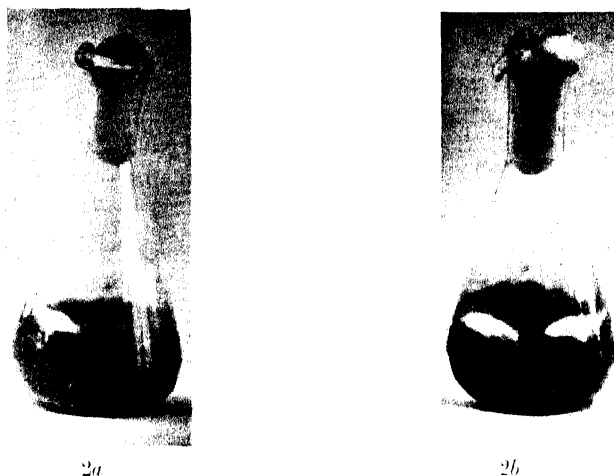
Isolate No. XXI was used in the cross-inoculation experiment. The six immature twigs of each of the six pear, peach, apricot and chestnut plants seedling stock (one year old) were cut aseptically with a pair of pruning scissors, inoculated with the mycellium of the fungus and covered over with cellophane bags. In the control, a drop of sterilized water was used instead of the culture. For the sake of comparison the culture of isolate XXI was inoculated on six immature twigs of apples in six plants. All the plants were kept inside a big glass cage and the inside of the cage was kept humid by spraying with water from time to time. The experiment was carried out on April 1938 and the infection was visible after a fortnight in all the inoculated twigs. In each case the fungus was reisolated on oatmeal agar and resembled the isolate culture in all respects. Thus it was proved that the strain No. XXI of *Botryosphaeria ribis* Gross. and Dugg. could easily infect through injured surfaces of twigs of pear, peach, apricot and chestnut. In some cases pycnidia-B appeared on the affected areas of the inoculated twigs.

#### SOURCES OF INFECTION

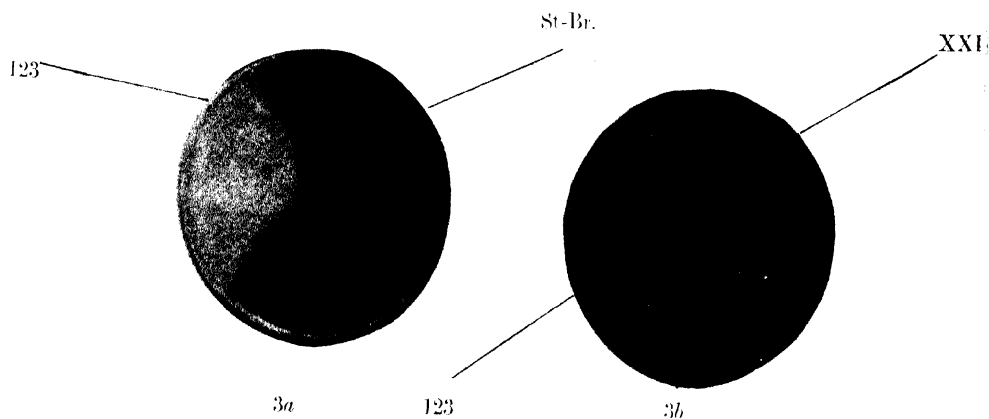
It was observed that on the already infected shoots pycnidial structures developed by the beginning of April but mature dothiorella type was not found till later. Mature pycnidial structures were only met with in the upper portion of diseased twigs, the lower portion having only immature pycnidia. In the latter part of May, and early June immature perithecia appeared in large numbers among the pycnidia. The dothiorella type of pycnidia, which was more numerous later on in the season continued to ooze out pinkish spore horns till the month of July. Ascospores appeared in June and were copious during the middle and later part of July. Field observations have shown that fresh infections through injured uncalled surfaces of stem and



1a-1c. Cultures No. XXI, St-Br and 123 growing on potato dextrose agar at 30°C. (3 month's old)



2a and 2b. Cultures No. XXI showing white patches of saltant No. XXIa (The culture was growing on rice meal agar at room temperature)



3a and 3b. Paired culture of No. XXI and 123 and St-Br. on Czapek's agar at 30°C. (18 days' old)



twigs occur in June and July when the spores were abundantly produced. The diseased areas became prominent in the following season. The spores seemed to be carried by rain water only. What part, if any, insects play in the dissimulation of spores was not observed. The pruned diseased twigs and stems left in the orchards also serve as a potent factor in the fresh infections.

#### METHODS OF CONTROL

As the fungus can only enter through the cut pruned surface of the apple stem, any suitable paint capable of preventing the entry of the fungus will be an effective method of controlling the disease.

##### *Experiments carried out in 1938*

In 1938 a control experiment was started to find out a suitable paint for covering the pruned surfaces of apple twigs as a protection against the entry of the fungus. This experiment was done in two sets—one for mature twigs and the other for immature ones. For each set 12 old trees were selected at random spread all over the orchard.

The following six treatments each replicated six times were carried out in each tree :—

- (i) Untreated (uninoculated) control I
- (ii) Untreated (inoculated) control II
- (iii) Treated with red lead-copper carbonate paste followed by inoculation. (The paste was prepared with 100 c.c. of raw linseed oil and 2 oz. each of red lead and copper carbonate.)
- (iv) Treated with red lead-Bordeaux powder paste followed by inoculation. (This paste was prepared with 75 c.c. of raw linseed oil and 2 oz. each of red lead and dried precipitate of 4 : 4 : 50 Bordeaux mixture.)
- (v) Treated with mercuric chloride solution followed by Bordeaux paste and then inoculated (the pruned surfaces were first wetted with 1/1000  $\text{HgCl}_2$  and after it dried they were painted with Bordeaux paste). (The Bordeaux paste was prepared by mixing 4 oz. of dried precipitate of 4 : 4 : 50 Bordeaux powder with 100 c. c. of raw linseed oil.)
- (vi) Treated with self-boiled lime-sulphur paste in raw linseed oil and then inoculated. (This paste was prepared with 4 oz. of self-boiled lime-sulphur, the proportion of quick lime and sulphur being 2 : 2 mixed in 100 c.c. of raw linseed oil.)

After the paints dried, the treated and untreated portions were inoculated six times with the culture of the fungus No. XXI, covered over with sterilized wet absorbant cotton and then with cellophane bags.

The inoculations were repeated from the middle of April to the middle of August at an interval of a fortnight. After about a month the twigs were examined in the laboratory.

The percentage of infection and the comparison of various treatments are given in Table I. From these tables it is evident that the paste of red lead and copper carbonate in raw linseed oil was the best paint for both mature and immature twigs, to prevent infection of their pruned surfaces.

TABLE I  
Comparison of different treatments (1938)

No.	Mean percentage of infection						General mean	Standard error of difference	Critical difference for significance	Whether significant by $\chi^2$ test
	(i) Untreated and uninoculated (control I)	(ii) Untreated and inoculated (control II)	(iii) Red lead and copper carbonate paste	(iv) Red lead and Bordeaux paste	(v) Bordeaux paste	(vi) Self-boiled lime-sulphur paste				
<i>(a) Immature twigs</i>										
1	Per treatment	43.05	86.10	31.94	65.27	74.91	61.55	6.28	13.3185488	Yes $P=0.01$
2	Percentage on general mean	69.90	139.88	51.89	106.04	121.69				
3	Percentage on control I	100.00	200.00	74.19	151.61	174.00				
4	Percentage on control II	50.00	100.00	37.09	75.80	79.03				
<i>(b) Mature twigs</i>										
1	Per treatment	90.27	100.00	31.94	90.27	93.04	83.09	8.77	7.3876492	Yes $P=0.01$
2	Percentage on general mean	108.64	120.35	38.44	108.64	111.97				
3	Percentage on control I	100.00	110.77	35.88	100.00	102.06				
4	Percentage on control II	90.27	100.00	31.94	90.27	93.04				

Dey and Singh [1939] found the paste of red lead with an equal amount of copper carbonate in raw linseed oil to be the best for controlling the stem-black disease of apple trees caused by *Coniothecium chomatosporum* Corda, which like the stem-brown disease always started from the pruned surfaces.

Thus by using the same paint it is possible to control both the stem-black and stem-brown diseases of apple trees.

#### *Experiments carried out in 1940*

In 1940, another control experiment was started with a view to finding out whether lanoline, which is known to hasten the formation of callus, is also effective in warding off the disease. Both mature twigs,  $\frac{1}{2}$  in. thick, and immature twigs of the apple variety Jonathan were used. Twenty-four trees, 12 for mature twigs and 12 for immature ones, were selected at random in the orchard. The following six treatments each replicated six times were carried out in each of the 12 trees :—

- (i) Untreated and uninoculated (control I)
- (ii) Treated with lanoline and uninoculated
- (iii) Treated with lanoline and inoculated
- (iv) Untreated and inoculated (control II)
- (v) Treated with lanoline mixed with an equal amount of red lead and copper carbonate and inoculated
- (vi) Treated with red lead and copper carbonate in equal amounts mixed with raw linseed oil and inoculated

After the paints dried the treated and untreated portions were inoculated with the culture of the fungus No. XXI, covered over with wet absorbant cotton and then with cellophane bags. Six inoculations were done after intervals of a fortnight. Twenty days after the last inoculation the twigs were examined in the laboratory.

The mean percentage of infection and the comparison of different treatments are given in Table II.

The following conclusions were arrived at :—

1. Treatment v (a paste of red lead and copper carbonate in equal amounts in lanoline) is significantly better than all the other treatments
2. Lanoline alone (treatments ii and iii) is unable to stop the entry of the fungus
3. Treatments i, ii, iii and iv are not significantly different from one another
4. Treatment vi (a paste of red lead and copper carbonate in raw linseed oil) is significantly better than treatments i, ii, iii and iv :—

(a)  $v > i = ii = iii = iv$

(b)  $vi > i = ii = iii = iv$

(c)  $v > vi$

#### DISCUSSION

This disease is primarily the disease of currants and was first noticed by Fairchild [1891] in the Hudson Valley. In 1896 the currant growers of Marlboo brought the disease to the attention of the New York Agricultural

TABLE II  
Comparison of different treatments (1940)

No.	Mean percentage of infection						General mean	Standard error of difference	Critical difference for significance	Whether significant by $\chi^2$ test
	(i)	(ii)	(iii)	(iv)	(v)	(vi)				
	Untreated and uninoculated (control I)	Treated with lanoline and uninoculated	Treated with lanoline and inoculated	Untreated and inoculated (control II)	Treated with lanoline mixed with red lead and copper carbonate in equal amounts and inoculated	Treated with red lead and copper carbonate in equal amounts in raw linseed oil and inoculated				
<i>(a) Immature twigs</i>										
1	Per treatment	97.22	98.61	100.00	98.61	38.88	82.17	5.53	$5.53 \times 1.95996 = 10.85788$	Yes $P=0.01$
2	Percentage on general mean	118.31	120.00	121.89	120.00	47.31				
3	Percentage on control I	100.00	101.42	102.86	101.42	39.99				
4	Percentage on control II	98.59	100.00	101.41	100.00	40.45				
<i>(b) Mature twigs</i>							77.76	6.82	$6.82 \times 1.95996 = 13.3669272$	Yes $P=0.01$
1	Per treatment	98.61	95.85	97.22	100.00	29.08				
2	Percentage on general mean	126.81	123.23	125.02	128.60	37.89				
3	Percentage on control I	100.00	97.18	98.59	101.41	37.91				
4	Percentage on control II	98.61	95.83	97.22	100.00	29.08				

Station. At this time Durand of Cornell University was investigating a similar currant disease found in western New York [Durand, 1897]. *Nectria cinnabarina* was named as the cause of the disease. In 1899 extensive observations carried out by Grossenbacher and Duggar [1911] established the fact that the currant blight occurring in the Hudson Valley is not caused by *Nectria cinnabarina* but by Fairchild's [1891] 'sterile' fungus. In 1907 the thorough study of the fungus was completed by Grossenbacher and Duggar [1911]. Putterill [1919] described a canker of apple trees in South Africa and named the fungus *Botryosphaeria mali* which differed from the currant fungus, *B. ribis*, in the width of its asci and the size of the stroma. Shear, Stevens and Wilcox [1925] believed that this fungus was not different from *B. ribis*.

Fenner [1925] described a fruit rot of apples caused by *B. ribis*. Birmingham [1924] described a canker of apple trees in South Wales. In 1934 Smith [1939] described the host range of the fungus and found that it included a list of 34 genera and 20 families of plants. This is a fungus of the type figured and described by Tulasne and Tulasne [1863] as *Dothidea melanops*, later on made into the type species of a new genus *Melanops* by Nitschke [1869]. Cesati and Notaris [1863] founded another new genus *Botryosphaeria* in which the forms like *D. melanops* were included, along with some phragmosporic forms. It is claimed, however, that since *Gibberella pulicares* (Fries) Sacc. may have been taken as a type species of *Botryosphaeria*, Nitschke's *Melanops* [1869] should be used for forms like the original *Dothidea melanops* Tul. However, Saccardo [1892] re-defined the limits of the genus *Botryosphaeria* by excluding the forms which develop septate spores. Even though the generic name *Melanops* may replace *Botryosphaeria* for fungi of this type because apparently the type species of the later was subsequently transferred to *Gibberella*, it seems unnecessary to revive an unused name for the isolated case involved in the investigation.

The presence of the two types of pycnidia and one type of perithecia in the life-history of the fungus makes the dissipation of the spores very active in causing primary infections. The comparative measurements of fructification from different hosts by various workers are given in Table III. From these comparative data it will be seen that the Indian strain very closely resembles *Botryosphaeria ribis* of Gross. and Dugg. The slight differences in the measurements of fructifications and spores may have been due to environmental differences.

#### SUMMARY

The stem-brown disease of apple is caused by *Botryosphaeria ribis* Gross. and Dugg., and usually starts from pruned surfaces and proceeds downwards causing a type of die-back.

Two types of pycnidia are found. Pycnidia of the *Sclerophoma* type are small and the pycnospores are bacillar, allantoid, and do not germinate. The other type of pycnidia are of the *Dothiorella* type, either single or in groups, and the pycnospores are fusoid to oblong, elliptic, hyaline to subhyaline, unicellular and germinate in tap water in 8-12 hours. The perithecia are found rarely in nature. These are either single or in groups of two to eight,

TABLE III  
Comparative sizes of fructifications of *Botryosporia* ribis from different hosts in different countries by different workers

Fungus	Host	Locality	Author	Pycnidia ( $\mu$ )	Pycnosporae ( $\mu$ )	Perithecia ( $\mu$ )	Asci ( $\mu$ )	Ascosporae ( $\mu$ )
<i>Botryosporia ribis</i> Gross. and Dugg.	Currant ( <i>Ribis vulgare</i> )	Hudson Valley, U. S. A.	J. G. Grossenbacher and B. M. Duggar [1911]	175—250 wide	18—31 $\times$ 4.5 —8	175—250 wide	80—120 $\times$ 17.20	16.23 $\times$ 5—7
<i>Botryosporia mali</i>	Apple ( <i>Pyrus malus</i> )	South Africa	V. A. Putterill [1919]	250 $\times$ 190	22.4 $\times$ 4.8	235 $\times$ 142 wide	96 $\times$ 13	19.2—19.5 $\times$ 6.5—8
<i>Botryosporia</i> sp. (Indian strain)	Apple ( <i>Pyrus malus</i> )	India, Chabhatia U. P., Kumaun Hills	U. B. Singh [1934]	126.0—294 $\times$ 140—199; Av. 220.7 $\times$ 166.2	9.1—25.9 $\times$ 6.3—7.7; Av. 17.52 $\times$ 6.77	140.0—252 $\times$ 180.2—280; Av. 192.0 $\times$ 190.7	36.4—112.0 $\times$ 14.0—18.9; Av. 77.49 $\times$ 15.39	11.9—28.0 $\times$ 8.4—12.6; Av. 20.1 $\times$ 10.7
<i>Botryosporia ribis</i>	Currant ( <i>Ribis vulgare</i> )	From various localities of U. S. A.	N. E. Stevens and A. E. Jenkins [1924]	...	14—31.5 $\times$ 4— 7.5 mostly 18.23 $\times$ 5.7	...	...	14.27 $\times$ 5.10 Av. 16.23 $\times$ 6.8

top shaped with ostioles. The asci are clavate, eight spored and hyaline. The ascospores are biserial, unicellular, hyaline, fusoid, elliptical to egg-shaped. Paraphyses are present and are filiform. These ascospores germinate readily in tap water in 10-14 hours.

Two isolates of the fungus were taken, one from a single pycnospore, the other from single ascospore. A third foreign strain was also included in the study. The cultural studies of the three strains were carried out on Czapek's agar, potato-dextrose agar and Brown's starch synthetic agar, and it was found that the strain XXI grew better on Brown's starch agar and the strain (St-Br) grew best on potato-dextrose agar. The macroscopic and microscopic observations were also recorded.

All these three strains were found to cause infection of apple twigs through both injured and uninjured surfaces. The progress of infection in all cases was very slow.

One of the three strains was cross-inoculated on cut twigs of pear, apricot, peach and chestnut and was found to infect them.

The cultural studies of the two saltants and the two parents were carried out on three different media—Brown's starch synthetic, cornmeal agar and potato-dextrose agar. The strain St-Br grew best in all three media; one of the saltants XXIa grew better than the parent and other saltant XXIa I, grew best in all the three media.

Pycnospores from the dothiorelia type of pycnidia and, to a lesser extent, ascospores carried by water serve as the potent factor for fresh infections. Fresh infections take place from May to July.

A mixture of red lead and copper carbonate in equal amounts in raw linseed oil when applied as a paste on the cut pruned surfaces of apple stem effectively controls the disease.

A paste of red lead and copper carbonate in equal amounts in lanoline is significantly better than a paste of red lead and copper carbonate in equal amounts in raw linseed oil.

#### ACKNOWLEDGEMENTS

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# CITRUS ROOTSTOCK TRIALS IN THE PUNJAB

## I. THE VIGOUR OF YOUNG TREES OF SWEET ORANGE, MANDARIN AND GRAPEFRUIT AS INFLUENCED BY DIFFERENT ROOTSTOCKS

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(With Plates XV-XVII and one text-figure)

THE most economic and widely accepted method of propagating citrus fruits both in India and elsewhere is by shield-budding a selected scion variety on a suitable rootstock. The success of the combination is markedly affected by both soil and climate, since it is well known that a given rootstock, which is satisfactory in one country or locality, may be a complete failure in another [Brown, 1920 ; Powell, 1930 ; Toxopeus, 1937 ; Webber, 1925]. Furthermore, certain rootstocks are known to exert a considerable influence on the vigour, cropping, etc. of the scion varieties budded or grafted on them [Brown, 1920 ; Bonns and Mertz, 1916 ; Batchelor and Webber, 1939 ; Hatton, 1927 ; 1928-29 ; 1931 ; 1935 ; Hodgson and others, 1937 ; Quinn, 1932 ; Rogers, 1926 ; Rogers and Vyvyan, 1928 ; Singh, 1936 ; Tydeman, 1926-27 ; Webber, 1934 ; Richards, 1938], and certain scions under appropriate conditions show an equally noticeable effect on certain rootstocks [Amos and others 1930 ; Brown, 1920 ; Halma, 1934 ; Hass and Halma, 1929 ; Hatton, 1923 ; 1927 ; Hodgson and others, 1937 ; Roberts, 1929 ; 1931 ; Swarbrick, 1927 ; 1931 ; Tukey and Brase, 1933 ; Webber, 1919 ; Wormald and Grubb, 1924 ; Vyvyan, 1930]. Recent rootstock investigations, especially in England and America, where considerable work has already been carried out on the problems arising directly from the twofold structure of a fruit tree, have tended to emphasize the need for standardized material for all types of investigation. In these countries the growers are at a much greater advantage than their contemporaries living in more backward places, for they will now plant only such scion-stock combinations as have been proved suitable to their conditions.

In our own country, excepting the preliminary work of Brown [1920], Shrivastava [1920] and Prayag [1920], little experimental evidence is available concerning rootstocks for use with the various cultivated species of citrus. Growers have vague and varied impressions of the so-called 'rootstock effect' and it is, therefore, not surprising that all sorts of rootstocks are in common use. The prevalence of low yields, inferior fruit quality and the early decline

of large number of trees is due in a large measure to the promiscuous use of miscellaneous rootstocks by Indian nurserymen.

The available evidence on rootstock investigations points to the fact that the performance of a budded or grafted plant is an expression of the reciprocal effect of the two symbionts. On this basis rootstocks have been designated as vigorous or dwarf, etc. but since the vigour of different scion varieties and species on the same rootstock is known to be different [Hatton, 1935 ; Roberts, 1929], the terms vigorous and dwarf must be regarded as purely relative. Not only this, the recent findings of various workers [Barker, 1927 ; Roberts, 1929 ; Singh, 1936 ; Tukey and Brase, 1933] show that a scion variety has apparently an inherent growth capacity which it normally exhibits, a capacity which may be dwarfed very considerably but which apparently may not be increased beyond a very small amount. This conclusion leads directly to the suggestion that in all cases, where rootstock influence is a limiting factor to tree size, it is of a dwarfing character. Roberts [1929] goes even as far as to suggest a classification of rootstocks on this basis. According to him the so-called vigorous rootstocks of Hatton [1926] are really neutral since they do not, in any way, limit the free development of the scion. Dwarfing rootstocks, on the other hand, are dominant since they do not allow the scion to develop to its natural capacity.

The present study forms part of a programme of rootstock investigations designed to determine the most suitable rootstocks for sweet orange (Malta), mandarin (Sangtra) and grapefruit in the Punjab, and was carried out during the years 1937-40 upon material planted at Montgomery in February, 1937.

The period of study covers only the vegetative phase of these trees. In view of the fact that growth responses may considerably change during development and maturity, it is essential that the evidence of rootstock influence concerning the two phases of the life of fruit trees should be discussed separately, and it is on this account that the results to date are reported at this stage.

#### EXPERIMENTAL MATERIAL

##### *Preparation of the material*

Nearly all the important varieties of citrus rootstocks were collected from various parts of India and Ceylon, partly to prepare the material for rootstock investigations and partly to make an enquiry into the distribution of different varieties of citrus so as to report on their nomenclature. The latter work will be dealt with in a separate communication.

Seeds as well as cuttings of the rootstock varieties were, as far as possible, obtained from the same parent tree in each case. Both seeds and cuttings were planted at the same time, viz. September 1932. Both the apogamic seedling rootstocks as well as those that could also be propagated by the rooting of stem cuttings were budded over in August 1935, at a uniform height of nearly 9 in. from the ground level, which is the practice commonly followed in the Punjab. It is obvious from the foregoing that the rootstocks, whether raised from seed or by the rooting of stem cuttings, were of uniform age at the time of budding, but it by no means follows that they were of the same vigour. Even within any given rootstock, the progeny differed considerably with respect to height and thickness. However, only fairly uniform

individuals in each lot were selected for budding and the variants were discarded as advocated by Webber [1920 ; 1932]. As a rule, nearly all the varieties made growth of sufficient vigour to allow of the budding operation, but some rootstocks definitely made much better growth than others.

The experimental material was prepared according to the established nursery practice followed all over the province. Seeds were sown in raised seedbeds in September 1932. The seedlings were dug up in September 1933 and the uniformly vigorous ones only were transplanted in nurserybeds. The stock became fit for budding in September 1935, viz. two years after transplanting, when uniformly vigorous seedlings were budded by a single operator. The budded nursery trees were transplanted in another piece of land at Lyallpur in September 1936 before finally planting the same in the orchard in February 1937 at Montgomery—a place about 200 miles from Lyallpur.

The transplanting of budded trees in the nursery, prior to final planting in the orchard, is not usually practised. But, in this particular case, the operation was considered necessary.

Three scion varieties, namely Malta local (*Citrus sinensis* Osbeck), Sangtra local (*C. nobilis* var. *deliciosa* Swingle) and grapefruit (*C. maxima* var. *livacarpa* Merrill and Lee) were budded in August 1935 on to each of the several rootstock varieties propagated from seed and cuttings. Although the preparation of genetically identical material could have been advantageously effected by placing scion buds gathered from a single tree on to carefully selected apogamic seedlings [Webber, 1932 ; Imp. Bar. Fruit Production Tech. Com., 1932], yet clonal vegetatively raised material was also used in order to answer such questions of practical and economic importance as (1) Will the vegetatively propagated rootstocks differ from the apogamic seedlings in their influence on the scion variety notwithstanding the similarity of their genetic build-up ? If so, how and to what extent and degree ? (2) How will the variability in the two sets of material as regards vigour, etc. determined at planting time compare later in the life-history of the experiment ? A further point of particular interest is the utility of the various rootstocks for Malta, Sangtra and grapefruit, under conditions similar to those where the experiments are conducted. This will be determined by investigations into the influence of rootstocks on scions as regards (a) growth and vigour, (b) productivity, (c) fruit quality, (d) resistance to diseases and (e) longevity. Information regarding all these points will be made available when the data collected is sufficient to justify their publication.

### *Description of the material*

As stated before, the experimental material broadly consists of two sets of groups. In one set, the three scion species are budded on to the several rootstocks raised from seed ; in the other set, the same three scion species are budded on to vegetatively propagated rootstocks. With a few exceptions, the rootstock varieties of the experimental material are the same in both the sets. For convenience of reference these sets will be designated as set A and set B. Set A constitutes the three scion-stock groups prepared by budding three scion species on several rootstocks propagated from seed, and set B includes the other three groups prepared by budding the same three

scion species on the rootstocks that were propagated by vegetative means. The specifying numbers allotted to the rootstocks in both sets of groups are purely arbitrary and are not based on any systematic study.

The various scion-stock combinations in sets A and B are tabulated in Table I.

TABLE I  
*Different scion-stock combinations under experiment*

Name of scion-stock combination					No. of trees under study	
Set A.—	(i) Malta local on rootstock No. 43 . . .				24	} 120
	Ditto 21 . . .				24	
	Ditto 20 . . .				24	
	Ditto 50 . . .				24	
	Ditto 9 . . .				24	
	(ii) Sangtra local on rootstock No. 43 . . .				18	} 90
	Ditto 9 . . .				18	
	Ditto 21 . . .				18	
	Ditto 20 . . .				18	
	Ditto 50 . . .				18	
	(iii) Grapefruit on rootstock No. 43 . . .				24	} 120
	Ditto 20 . . .				24	
	Ditto 47 . . .				24	
	Ditto 50 . . .				24	
	Ditto 9 . . .				24	
Set B.—	(i) Malta local on rootstock No. 20 . . .				18	} 72
	Ditto 43 . . .				18	
	Ditto 50 . . .				18	
	Ditto 9 . . .				18	
	(ii) Sangtra local on rootstock No. 43 . . .				18	} 72
	Ditto 20 . . .				18	
	Ditto 9 . . .				18	
	Ditto 50 . . .				18	
	(iii) Grapefruit on rootstock No. 43 . . .				18	} 72
	Ditto 20 . . .				18	
	Ditto 50 . . .				18	
	Ditto 9 . . .				18	

The material in sets A and B is planted separately in contiguous fields but the lay-out, viz. arrangement of plots, position of main water channels, block water channels and position of paths, etc. is uniform in each case.

#### LAY-OUT

It is evident from the foregoing section that sets A and B have each three different groups of scion-stock combinations, depending upon the number of scion varieties employed. There are thus in all six different groups of

experimental material planted in six different fields. The randomized block method, being most adaptable to field experiments in horticulture, is the one here adopted. The arrangement and distribution of different scion-stock combinations within the plots is explained by Fig. 1. The method of lay-out of each field is the same as shown in this figure.

A glance at the lay-out, given in Fig. 1, shows that there are seven rootstocks under trial in set A, of which a comparative study of only five is reported in this paper. The position of the trees on rootstocks 44 and 1, which have not been dealt with, is shown by shaded sub-plots in the figure. These two rootstocks have turned out to be horticulturally the same as No. 20 (rough lemon). Thus, rough lemon is represented thrice in each replicate, whereas each of the four remaining rootstocks is represented only once in each block. With a view to keeping uniformity in the number of replications (one sub-plot to represent each treatment in each block) it was necessary to select one out of the three identical varieties originally planted; No. 20 was selected because in set B, rootstocks 44 and 1 were not included for trial and only 20 was used.

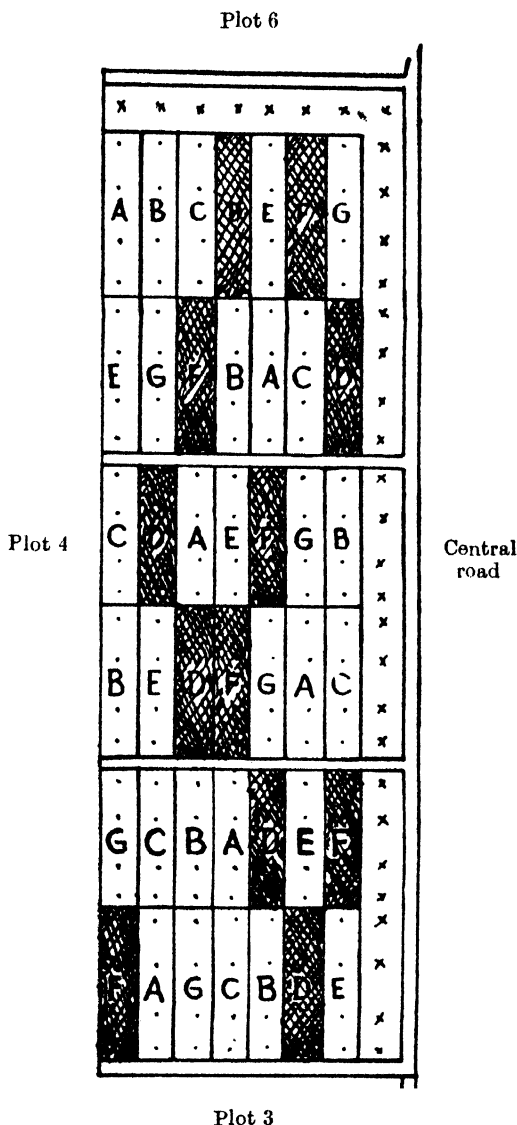


FIG. 1. The distribution of seven scion-stock combinations in six blocks in plot No. 5

- |                                     |                                   |
|-------------------------------------|-----------------------------------|
| A. Malta local on rootstock No. 20* | B. Malta local on rootstock No. 9 |
| C. " " No. 21                       | D. " " No. 1*                     |
| E. " " No. 43                       | F. " " No. 44*                    |
| G. Malta local on rootstock No. 50  |                                   |

..... Experimental trees      × × × × × Non-experimental trees  
 ————— Water channels

\*Rootstocks No. 20, 1 and 44 are found to be one and the same.

The piece of land for each experiment is divided into six blocks of similar dimensions. Thus, six replications are provided for each series of scion-stock combination. Each block in turn is divided into as many plots of equal size and shape as the number of rootstock varieties to be compared. The allocation of scion-stock combinations to particular plots was determined by drawing lots, as a result of which each combination is represented once in each block. The dimensions of the plots vary from 20 ft.  $\times$  60 ft. to 20 ft.  $\times$  80 ft. depending upon the number of trees planted. In other words, a unit of three to four trees of any particular scion-stock combination is replicated six times, so that there are 18-24 trees of this in any one experiment.

The planting of all the six experimental fields follows the square system. A uniform distance of 20 feet from tree to tree and row to row is being adopted.

Due attention was given to the variability within the experimental material at the time of planting. The trees within each lot were roughly graded according to vigour and planted in such a way as to include only fairly uniform ones of all combinations in each block. There being six blocks placed end to end in each case (Fig. 1), the variability within the material is allowed for in block variance when computing the data.

#### METHODS EMPLOYED

Soon after planting the material in 1937, trunk circumference measurements of all the trees were taken at a uniform height of approximately one foot from the ground level. The places of measurement were marked with white paint so that the girth measurements could be taken at a fixed point year by year. The union of the scion with the rootstock was invariably below the place of measurement.

The girth measurements were taken again in 1938, 1939 and 1940 at yearly intervals with a view to determining the growth response during early years of the various scions growing on different rootstocks. The girth measurements serve as a measure of the vigour of the trees in these trials. The data regarding girth, for all the years, have been examined statistically by the analysis of variance [Fisher, 1934]. The differences above the 5 per cent level only are taken as significant. On the basis of the information thus gathered the various scion-stock combinations have been grouped so as to show at a glance their behaviour from year to year.

#### DISTINGUISHING NAMES OF THE ROOTSTOCK VARIETIES EMPLOYED

The morphology and other characters of the rootstock varieties employed in this study along with others in the collection have been studied during the last two years in order to clarify their nomenclature. This work, when completed, will be published separately, but in the meantime the rootstocks under trial have been properly identified as follows:—

TABLE II

*Distinguishing names of the rootstock varieties under trial*

Names under which received	Arbitrary specifying numbers allotted as referred to in this paper	Popular local names in the Punjab	English equivalents	Specific names
1. <i>Mitha</i> . .	50	<i>Mitha</i> .	Sweet lime .	<i>C. aurantifolia</i> var. Swingle
2. <i>Khatti</i> . .	20	<i>Jatti khatti</i>	Rough lemon	<i>C. limonia</i> Osbeck
3. <i>Chakotra</i> .	47	<i>Chakotra</i> .	Shaddock .	<i>C. maxima</i> Merrill
4. <i>Turanj</i> .	9	<i>Mokari</i> .	Citron . .	<i>C. medica</i> Linn.
5. <i>Kharna khatta</i>	43	<i>Kharna khatta</i>	Nil	<i>C. karna</i> Raf.
6. <i>Nasnaran</i> .	21	Nil	Nil	<i>C. japonica</i> Thumb

Beside the six rootstocks mentioned above, another apparently distinct species, viz. *khatta* (*C. aurantium* Linn.) was also included, but on studying its morphological characters it was found to be really rough lemon. It is evident that the material used as rootstocks does not include species like *Citrus aurantium*, *C. nobilis* and *C. sinensis*, etc. These species, especially the first one, are probably indigenous to India, and may, therefore, have great possibilities as rootstocks in view of the fact that they are already in use in some of the citrus-growing countries of the world [Coit, 1927 ; Hume, 1930 ; Powell, 1930]. In preparing further material, these species will be included as well as others that are now growing in the rootstock collection plot at Montgomery. Furthermore, certain points of practical importance regarding these rootstocks are being studied in preparation to a further and more extended trial of rootstock material.

## PRESENTATION OF RESULTS

As mentioned in previous sections, the girth measurements of all the trees constituting the various scion-stock combinations were taken annually (1937-40) as an index of their vigour as influenced by the rootstocks on which they were stem-budded. The data for 1937 relates to the behaviour of maidens as at the time of planting in the orchard, that for 1938 as the behaviour of one-year old plants and that for 1940 as the behaviour of three-year old plants. The material constitutes two sets, set A and set B. In set A apogamic seedlings have been used as rootstocks and in set B the rootstocks used were propagated by the rooting of stem cuttings. The genetic composition of any particular rootstock in both the sets is thus identical but apogamic seedlings

and rooted cuttings being physiologically different, the results of their performance with different scions are presented separately as under :—

*Scion-stock combinations of set A with different scion varieties*

1. *Malta local*.—The mean data and significant difference at the 5 per cent level are compiled in Table III which shows the relative size of three-year old plants of this variety as influenced by different rootstocks (Plates XV-XVII).

TABLE III

*Performance of Malta local variety with certain rootstocks of apogamic origin*

Year	Average circumferential measurements in cm. for rootstock numbers					General mean	S. E.	Significant difference for $P=0.05$
	43	21	20	50	9			
1937 . . .	5.08	5.63	3.55	3.35	3.61	4.25	0.23	0.72
1938 . . .	5.83	6.29	4.28	4.06	3.99	4.88	0.29	0.91
1939 . . .	9.97	8.83	8.50	7.73	6.11	8.23	0.57	1.80
1940 . . .	16.7	15.7	15.4	14.0	13.2	14.97	0.66	2.1

The performance of different scion-stock combinations has first to be examined separately for all the four years before discussing them together. The resultant effect of the various combinations on the vigour of the scion is tested by comparing the difference between average circumferential measurement figures relating to any pair of combinations with the significant difference figure given in the last column for each year. On the basis of this comparison, the various rootstocks, differentiating themselves from one another, are put in different groups. When certain rootstocks are found to exhibit only small differences as compared with the significant limit, they are put in one and the same group. The grouping of the various rootstocks, based on the statistical examination of the data given in Table III, is made as under :—

Year	Group 1	Group 2
1937 . . .	21 ; 43	9 ; 20 ; 50
1938 . . .	21 ; 43	20 ; 50 ; 9
1939 . . .	43 ; 21 ; 20	50 ; 9
1940 . . .	43 ; 21 ; 20	50 ; 9

The figures show that the above five rootstock varieties fall into two groups even in the nursery stage. Thus, rootstocks 21 and 43 are associated with the more vigorous trees of Malta local variety, whereas the remaining rootstocks (in group 2) are associated with trees of medium vigour. The grouping of the rootstocks for 1938, viz. one year after planting, was substantially the same except in their order of vigour within group 2. By the end of the second year of their life in the orchard, i.e. in 1939, the various rootstocks still fall into two groups but their order of vigour is considerably changed. Thus, rootstock 20 is now bracketed with 43 and 21 as being vigorous, and trees

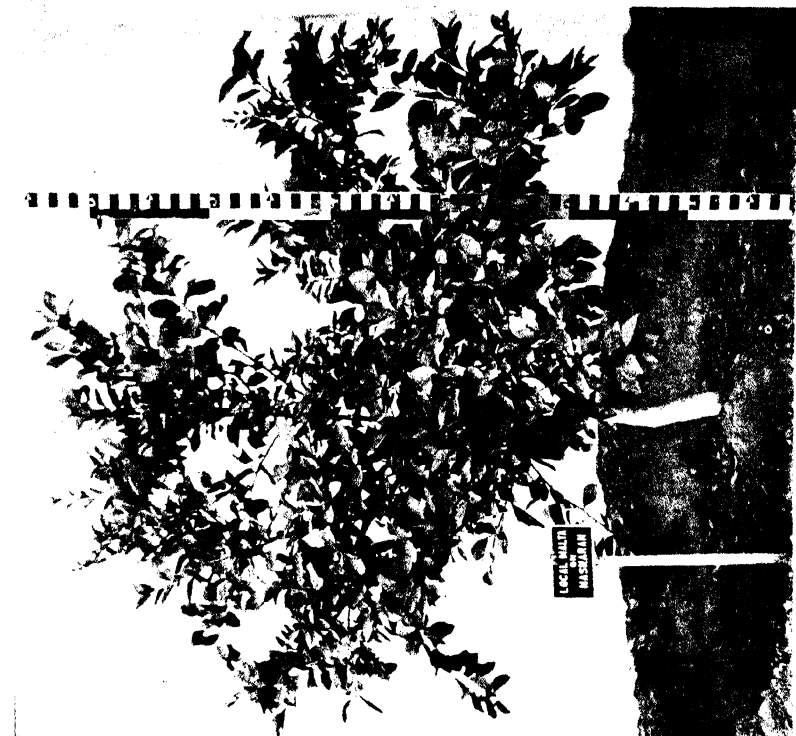


FIG. 2. Local Malva on *khajana*



FIG. 1. Local Malva on *khajana*

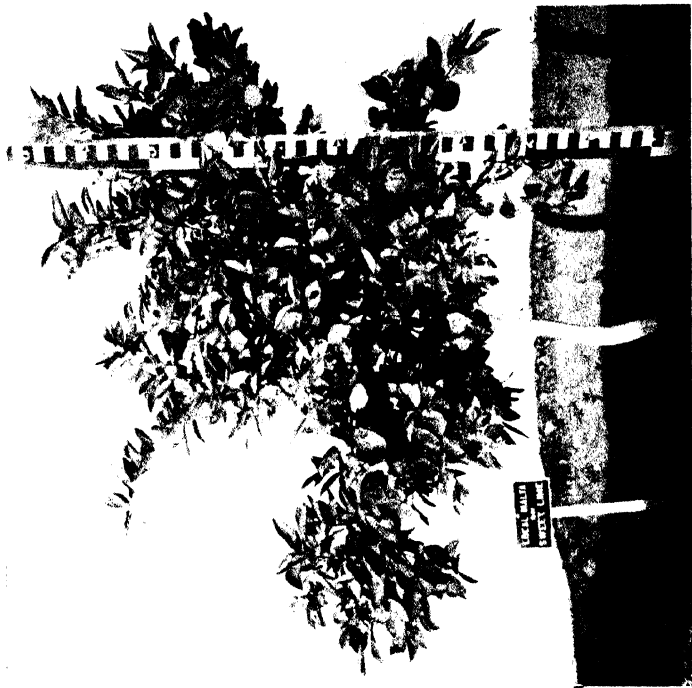


FIG. 2. Local Malta on *mitha*



FIG. 1. Local Malta on *jatti khatti*

on stock No. 43 are now slightly more vigorous than those on No. 21. Rootstocks 50 and 9, however, remained in group 2. The behaviour of the different rootstocks in 1940 remained exactly the same as during 1939 and it appears that rootstocks 50 and 9 may ultimately prove to be dwarfing for the Malta local variety.

2. *Sangtra local*.—The mean data and significant difference at the 5 per cent level are compiled in Table IV, which shows the relative size of three year old trees of this variety as influenced by different rootstocks.

TABLE IV

*Performance of Sangtra local variety with certain rootstocks of apogamic origin*

Year	Average circumferential measurements in cm. for rootstock numbers					General mean	S. E.	Significant difference for $P=0.05$
	43	9	21	20	50			
1937 . . .	4.97	3.42	3.19	2.17	2.33	3.22	0.18	0.55
1938 . . .	6.14	4.53	4.16	2.83	3.38	4.21	0.17	0.53
1939 . . .	11.77	9.34	8.61	7.26	7.96	8.99	0.30	0.94
1940 . . .	19.5	16.1	15.9	15.5	14.5	16.28	0.41	1.32

The grouping of the various rootstocks, based on the statistical examination of the data given in Table IV above, is made as under :—

Year	Group 1	Group 2	Group 3	Group 4
1937 . .	43	9; 21	50; 20	..
1938 . .	43	9; 21	50	20
1939 . .	43	9; 21	50; 20	..
1940 . .	43	9; 21; 20	50	..

The above grouping shows that with this variety rootstock No. 43 (*kharna khatta*) is differentiated from the rest in that the trees of the Sangtra local variety are extremely vigorous even from their nursery stage and that this vigour has been maintained throughout the period covered by this report. Next in order of vigour are trees on rootstocks 9 and 21— an order which remained unchanged for all the four years. By 1940, however, one more rootstock, viz. No. 20, has jumped up in this group. In the third and fourth groups, the various rootstocks did not materially change their order of vigour for the first three years but by 1940, No. 50 alone remained in the third group. This rootstock may, therefore, ultimately prove to be a dwarfing rootstock for Sangtra local scion variety.

3. *Grapefruit (Marsh Seedless)*.—The mean data and significant difference at the 5 per cent level are compiled in Table V, which shows the relative size of three-year old trees of this variety as influenced by different rootstocks.

TABLE V

*Performance of grapefruit (Marsh Seedless variety) with certain rootstocks of apogamic origin*

Year	Average circumferential measurements in cm. for rootstock numbers					General mean	S. E.	Significant difference for $P=0.05$
	43	20	47	50	9			
1937 . . .	3.99	3.72	3.75	3.19	2.82	3.50	0.158	0.50
1938 . . .	5.43	5.43	5.02	4.42	4.25	4.91	0.208	0.66
1939 . . .	10.36	10.18	9.27	8.06	8.30	9.18	0.335	1.09
1940 . . .	18.7	18.8	16.6	14.7	12.4	16.12	0.477	1.50

The grouping of the various rootstocks, based on the statistical examination of the data given in Table V above, is made as under :—

Year	Group 1	Group 2	Group 3	Group 4
1937. . .	43 ; 47 ; 20	50 ; 9	..	..
1938. . .	43 ; 20 ; 47	50 ; 9	..	..
1939. . .	43 ; 20 ; 47	9 ; 50	..	..
1940. . .	43 ; 20	47	50	9

It is evident that, in 1937, with grapefruit as the scion variety, the three rootstocks Nos. 43, 47 and 20 are associated with more vigorous trees, while the remaining two, viz. 50 and 9, formed a class which proved to be less vigorous. The relative performance of the three vigorous rootstocks did not change in the following year except that the order of vigour of stocks 47 and 20 in group 1 is reversed. A year later, viz. the beginning of 1939, the order of vigour of rootstocks in group 1 still remained unchanged, although a slight change occurred in group 2. By the beginning of 1940, however, the five rootstocks under trial differentiated into four different groups with regard to their influence on the vigour of grapefruit scion. Thus, grapefruit trees on stocks 43 and 20 are of greatest vigour, those on 47 are of medium vigour, while those on 50 and 9 are dwarf notwithstanding the fact that No. 9 is significantly more dwarfing than No. 50.

#### *Scion-stock combinations of set B with different scion varieties*

1. *Maita local*.—The mean data and significant difference at the 5 per cent level are compiled in Table VI, which shows the relative size of three-year old trees of this variety as influenced by different rootstocks.



*Local Malta on mokari*



TABLE VI

*Performance of Malta local variety with certain rootstocks raised by the rooting of stem cuttings*

Year	Average circumferential measurements in cm. for rootstock numbers				General mean	S. E.	Signifi- cant dif- ference for $P=0.05$
	20	43	50	9			
1937 . . . . .	4.08	4.65	5.36	3.60	4.38	0.357	1.12
1938 . . . . .	6.00	6.09	6.66	4.74	5.89	0.30	0.94
1939 . . . . .	11.49	11.39	10.63	8.36	10.47	0.36	1.14
1940 . . . . .	19.1	19.0	16.4	13.1	16.91	0.46	1.47

The grouping of the various rootstocks, based on the statistical examination of the data given in Table VI, is made as follows :—

Year	Group 1	Group 2	Group 3
1937 . . . . .	50; 43	20; 9	..
1938 . . . . .	50; 43; 20	9	..
1939 . . . . .	20; 43; 50	9	..
1940 . . . . .	20; 43	50	9

The grouping of rootstocks shows that at the nursery stage in 1937, they fall into two groups only. In group 1 occur the rootstocks 50 and 43 which are associated with more vigorous trees of the Malta local scion. The trees on rootstocks 20 and 9 form a less vigorous class. One year after planting, viz. in 1938, rootstock 20 had moved from group 2 into group 1, but the order of vigour of the two rootstocks in group 1 did not change. In 1939, the order of vigour of rootstocks in group 1 changed considerably. Thus, rootstock 50, which occupied 1st position in 1937 and 1938, went to the last position and rootstock 20, which occupied last position in 1938, came to the foremost rank. However, one should not lose sight of the fact that, despite the change in the order of vigour of these rootstocks, they are statistically identical, but this abrupt behaviour of stock No. 50 promises further segregation among the varieties in this group. Rootstock No. 9, however, remained in group 2 as before. In 1940, rootstock 50 dropped back to group 2 but the remaining two stocks in group 1 maintained their performance. It cannot be foretold which of the two rootstocks in group 1 will ultimately prove to be the most vigorous, but it would appear that stock No. 9 is likely to prove a dwarfing stock for this scion variety more so than any other, and that stock No. 50 may also prove dwarfing to this variety.

2. *Sangtra local*.—The mean data and significant difference at the 5 per cent level showing the relative size of three-year old trees of this variety as influenced by different rootstocks are compiled in Table VII.

TABLE VII

*Performance of Sangtra local variety with certain rootstocks raised by the rooting of stem cuttings*

Year	Average circumferential measurement in cm. for rootstock numbers				General mean	S. E.	Significant difference for $P=0.05$
	43	20	9	50			
1937 . . . . .	5.76	2.39	2.48	4.23	3.72	0.301	0.95
1938 . . . . .	7.09	4.20	4.07	5.78	5.29	0.468	1.48
1939 . . . . .	13.24	9.71	10.07	11.02	10.99	0.335	1.06
1940 . . . . .	21.1	16.9	16.8	16.6	17.8	0.48	1.5

The grouping of various rootstocks, based on the statistical examination of the data given in Table VII, is made as follows :—

Year	Group 1	Group 2	Group 3
1937 . . . . .	43	50	9; 20
1938 . . . . .	43; 50	..	20; 9
1939 . . . . .	43	50; 9	20
1940 . . . . .	43	20; 9; 50	..

The data show that throughout the four-year period, trees of Sangtra scion variety on rootstock No. 43 are more vigorous than on the remaining three rootstocks. Rootstock No. 50, which proved next in order of vigour during the first three years, had, by the end of the fourth year, proved a dwarfing rootstock. No. 20, on the other hand, which was dwarfing as compared with others during the first three years, was eventually shown to have an invigorating effect. At the end of four years, Nos. 20, 9 and 50 are not significantly different from one another and, as a class, they are significantly less vigorous than No. 43.

3. *Grapefruit (Marsh Seedless)*.—The mean data at the 5 per cent level are compiled in Table VIII which gives the relative size of three-year old trees of this variety as influenced by different rootstocks.

TABLE VIII

*Performance of grapefruit (Marsh Seedless) with certain rootstocks raised by the rooting of stem cuttings*

Year	Average circumferential measurements in cm. for rootstock numbers				General mean	S. E.	Significant difference for $P=0.05$
	43	20	50	9			
1937 . . . . .	6.11	3.65	4.88	4.15	4.70	0.324	1.03
1938 . . . . .	7.75	5.50	5.71	5.61	6.13	0.380	1.21
1939 . . . . .	12.94	11.39	10.04	9.77	11.03	0.4	1.28
1940 . . . . .	21.2	20.3	15.8	15.5	18.23	0.375	1.2

The grouping of various rootstocks, based on the statistical examination of the data given in Table VIII, is made as follows :—

Year	Group 1	Group 2	Group 3
1937 . . . . .	43; 50	9; 20	..
1938 . . . . .	43	50; 9; 20	..
1939 . . . . .	43	20	50; 9
1940 . . . . .	43; 20	..	50; 9

At the time of planting, i.e. at one-year old, the rootstocks 43 and 50 were both associated with vigorous trees of the Marsh Seedless grapefruit variety, while the remaining rootstocks, viz. 9 and 20, formed a second class which differed from them in this respect. By the end of the next year, rootstock No. 43 alone remained in the vigorous group, and the remaining three fell into group 2. In 1939, viz. two years after planting in the orchard, a further differentiation took place in the rootstocks of group 2, by which trees on No. 20 became significantly more vigorous than those on either of No. 50 or 9. By 1940, trees on rootstock No. 20 became still more vigorous, and they are now nearly as vigorous as those on rootstock No. 43, whereas rootstocks No. 50 and 9 appear to be dwarfing ones.

#### DISCUSSION

##### 1. Rootstock No. 43

The performance of rootstock No. 43 [*Kharna khatta* (C. karna Raf.) synonyms : *Id lemon* (Poona, Bombay), *Soh sarakar* (Assam), *Mokari* (Renala Khurd, Punjab) is outstanding in that it is associated with the most vigorous trees of all the three scion varieties budded on it. This is true for both seedling and cutting material. It differentiates, in this respect, from most of the remaining rootstocks as early as one year after budding. The vigorous growths of trees on this rootstock, coupled with the fact that its seedlings are usually fit for budding earlier than those of other varieties, indicate that, from the nurseryman's standpoint, it is a rootstock which may become widely used because, other things being equal, good nursery trees of a given scion variety can be produced relatively early. Furthermore, the consistent behaviour of scions on this rootstock for the first four years after budding strengthens the view that, as compared with other varieties included in these trials, this one may ultimately produce trees of the largest size. This point is of special significance in so far as it holds good for all the three scion varieties employed.

Brown [1920] reported a similar invigorating influence of *kharna* rootstock which, according to the photograph of fruit in Plate VI, fig. 1 presented by him, appears to be of the same variety as *kharna khatta* employed in the present investigations. Brown, however, translated this as rough lemon, a variety from which it differs in almost every respect. It would appear, therefore, that Brown did not employ the true rough lemon in his trials, although he has reported the results of *kharna khatta* as being due to rough lemon. The results of Brown have also been quoted by subsequent workers [Gardner *et al.*, 1922] as the effects of rough lemon rootstock upon scion growth, a repetition of which should be avoided.

So far as the present authors are aware, *kharna khatta* as a rootstock has not been employed in other citrus-growing countries of the world, and it is very probable that this species is a native of India. Its performance in these trials warrants an extension of its use on an experimental scale in this country and elsewhere.

## 2. Rootstock No. 20

The use of this rootstock [*jatti khatti* or rough lemon (*C. limonia* Osbeck), synonyms : *Khatta* (Renala Khurd and Lahore, Punjab) *Khatti* (Lyallpur and Shahdara, Punjab)] has been investigated in almost every citrus-growing country of the world. It should, however, be clearly understood that this rootstock was not employed by Brown [1920]; Shrivastava [1920] and Prayag [1920] in their investigations in North-West Frontier Province, Central Provinces and Bombay and that *Id lemon* and *jamburi* said to be the varieties of rough lemon [Imp. Bur. Fruit Production, Tech. Com., 1932] have nothing in common with the real rough lemon of South Africa, California and Florida, etc. [Coit, 1927; Hume, 1930; Powell, 1930]. It follows, therefore, that no work has yet been done anywhere in India to elucidate the influence of rough lemon on the commercially grown citrus scion varieties of this country.

As a result of the satisfactory growth of trees on rough lemon in most parts of the citrus world, it is the one most used in South Africa [Marloth, 1938; Powell, 1930]. In Arizona, California and Java [Coit, 1927; Marloth, 1938; Toxopeus, 1936] it is used to a limited extent. In Florida [Batchelor and Webber, 1939; Coit, 1927; Hume, 1930; Marloth, 1938] it is the main rootstock for very sandy soils although, according to Davis [1928], it thrives equally well on heavy loams. In Dominica, rough lemon has proved to be a good stock for acid limes [Imp. Bur. Fruit Product. Tech. Com., 1932]. In Australia [Marloth, 1938] it is employed extensively, and in the Punjab it is also one of the widely used rootstock varieties. Cheema [1929] advises rough lemon as the most suitable rootstock for lemons in western India, though the species actually used is different from real rough lemon. It is evident from the above, therefore, that with rough lemon the problem appears to be one of adaptability to local soil and climatic conditions rather than congeniality between stock and scion, since cases of absolute failure or imperfect union have not been reported from anywhere.

The data presented in the foregoing section show that rough lemon is a promising rootstock and its growth influence in case of the sweet orange (Malta) and grapefruit scions may ultimately be in line with *kharna khatta*, but in the case of Sangtra scion variety it may not prove equally vigorous. It is, however, significant that even with sweet orange and grapefruit scions, it did not produce as vigorous nursery trees in the same period as was done by some other rootstocks. All the same, the experience of workers in other countries [Coit, 1927; Hume, 1930], that this rootstock is associated with vigorous vegetative growth of young trees, is also corroborated by the data here reported.

## 3. Rootstock No. 50

Sweet lime [*mitha* (*C. aurantifolia* var. Swingle)] is, perhaps, indigenous to India. On its own roots, it is quite vigorous and spreading but when used

as a rootstock for sweet orange, mandarin and grapefruit, the resulting trees are dwarfed. It is a favourite with nurserymen throughout the Punjab, partly due to the prevalent belief that it improves the texture and quality of fruit of the scion varieties worked on it, and partly to its ability to root readily from stem cuttings producing well-grown nursery stock in a comparatively short period. The latter point concerns mainly the nurseryman, who is chiefly interested in the production of plants in a short space of time, but the former point concerns the grower and the fruit industry in general and is, therefore, more worthy of consideration and experimentation.

The data show conclusively that, with all the three varieties in set A, sweet lime rootstock behaves as a dwarfing type. In set B, however, the trees on this stock are vigorous when young, but by the beginning of 1940, the trees on some other rootstocks had significantly outgrown them. The difference in the vigour of scion varieties in sets A and B, brought about by this rootstock for the first few years was, therefore, of a transitory nature and was mainly due to the increased vigour of nursery stock in set B at the time of budding. Evidently therefore the nursery stock of sweet lime, raised by the rooting of cuttings, became fit for budding comparatively much earlier than that raised from seed. Not only this, with the exception of *galgal* (hill lemon) which is not employed in these trials, sweet lime cuttings resulted in nursery stock of a better size and vigour than that of the remaining stock varieties under trial in set B. Since time of budding was the same in sets A and B, the stocks in set B made much better growth than those in set A and even within set B the sweet lime stock made better growth than others in view of its better rooting capacity and was consequently at an advantage to induce better growth of scion buds placed in it. This initial advantage, however, was not maintained and this rootstock must now be regarded as dwarfing in character as in the case of set A. All the three scion varieties worked on sweet lime in sets A and B are somewhat dwarfed, and these effects are in general agreement with the results obtained by Brown [1920] in India and are also in accord with the experience of growers in south-eastern Mediterranean countries, especially in Palestine [Hodgson, 1931; Powell, 1930], where Jaffa or Shamouti variety of sweet orange is mainly grown on sweet lime.

#### 4. Rootstock No. 21

According to Bonavia [1880] this rootstock [*nasharan* (*C. japonica* Thumb)] resembles in character the *reshmi* orange of the United Provinces, India, and is not a true mandarin although it is called mandarin in Ceylon. It was introduced into the Punjab through the courtesy of the Curator, Royal Botanic Gardens, Paradeniya, Ceylon, and was included only in set A (rootstocks of apogamic origin) as the cuttings did not root well. On its own roots, this rootstock is comparatively dwarf, but when used as a rootstock for sweet orange (Malta local) it has produced maidens of greater vigour than those of the most vigorous known rootstocks. On transplanting to the orchard, the trees on this rootstock naturally received the greatest setback and some of them even died, but the remaining ones are now growing well. On the other hand, when used as a rootstock for mandarin (Sangtra) it produced maidens of medium vigour only, which when transplanted grew very well indeed. Furthermore, the results (Tables III and IV) show that, both with sweet orange and mandarin as

the scion varieties, this rootstock maintained its nursery performance, viz. that of producing vigorous trees in case of the former and dwarf trees in case of the latter throughout the course of these investigations. This differential response may be partly correlated with the fact that the union of this rootstock with Malta scion is perfect, but with Sangtra scion the stock stem outgrows the scion stem.

#### 5. \* Rootstock No. 9

This species [*mokari* or citron (*Citrus medica* Linn.), synonyms : *Turanj* (Renala Khurd, Punjab), *Sak limboo* (Poona), *Nattaran* (Ceylon), *Sohmad* (Assam)] is a native of India [Powell, 1930]. It has two forms : one where the rind is very rough, corrugated and is orange on ripening (C. Mahalung, Poona) and the other where the rind is smooth and is yellow on ripening. It is the latter form that has been employed in the trials reported here.

The nursery performance of this species is equal to that exhibited by our best rootstocks. It is easily grown from cuttings and both cutting and seedling stocks reach the budding stage as quickly as those of any other stock. Probably the greatest point in its favour is the fact that variability in the seedbed with regard to the height and vigour of seedlings is low and in consequence only a small percentage of seedlings has to be discarded at the transplanting time. The main disadvantage appears to be its extreme susceptibility to citrus canker and gum diseases.

Out of the three scion varieties, viz. sweet orange (Malta local), mandarin (Sangtra local) and grapefruit, with which it has been tried as a rootstock, it has given good union only with mandarin while in the remaining two cases the scion portion has invariably outgrown the rootstock.

The data show conclusively that with sweet orange and grapefruit, where it is not quite compatible, its effect is that of dwarfing nature but in case of Sangtra local scion the trees on this rootstock are fairly vigorous. In this respect, therefore, like *nasnaran* it has responded differently with different scion varieties. It has been largely used as a rootstock in Egypt [Brown, 1936] with the result that trees budded or grafted on it remain inferior in vigour and resistance to diseases. This experience of Egyptian growers is in conformity with the results obtained in the Punjab.

#### 6. Rootstock No. 47

As far as could be ascertained, shaddock [*chakotra* (*Citrus maxima* Merrill)] has never been employed as a rootstock in the Punjab for orange, mandarin and grapefruit. However, in view of the remarks of Webber [1925] that shaddock has been favourably commented on as a rootstock for grapefruit in South Africa, it was considered desirable to include this in the citrus rootstock trials, especially in the case of grapefruit.

It has made a good union with grapefruit and the results show that trees on this stock are fairly vigorous. Although the trees have only completed three years in the orchard, some of them growing on this rootstock are severely affected with mottle leaf. Whether it is purely accidental that the portions of the land, on which the trees of this scion-stock combination are planted, are deficient in certain elements causing mottle leaf or whether this rootstock itself is responsible for this malady cannot at present be determined.

## SUMMARY

The influence of certain rootstocks on the vigour of young stem-budded scions of sweet orange (Malta), mandarin (Sangtra) and grapefruit has been studied at Montgomery, Punjab, for the years 1937-40.

The results show that:—

- (a) *Kharna khatta* (*C. karna* Raf.) is associated with the most vigorous trees irrespective of the scion variety budded on it.
- (b) *Jatti khatti* or rough lemon (*C. limonia* Osbeck) is also associated with vigorous-growing trees, but it does not equal *kharna khatta* in this respect.
- (c) *Mitha* or sweet lime (*C. aurantifolia* var. Swingle) appears to be a dwarfing rootstock for each of the three scion species budded on it.
- (d) *Nasnaran* (*C. japonica* Thumb) and *mokari* or citron (*C. medica* Linn.) both appear to be variable in their influence as rootstocks. For instance, sweet orange on *nasnaran* has proved vigorous, but mandarin trees on this rootstock are decidedly dwarfed. Similarly, plants of sweet orange and grapefruit are dwarf on *mokari*, but those of mandarin on the same rootstock are vigorous.
- (e) Grapefruit trees on *chakotra* or shaddock (*C. maxima* Merrill) show mottle leaf but it has not yet been determined whether this is due to soil or to rootstock.

The vigour of different scion species has been shown to be variable on one and the same rootstock in case of two rootstock species, namely *nasnaran* and *mokari*. The terms vigorous and dwarf usually assigned to rootstocks should be used only in connection with specified scion varieties.

The vigour of an unworked rootstock is no criterion of its vigour as a rootstock when grafted with certain scion varieties. For instance, *nasnaran* which is comparatively dwarf on its own roots has, when used as a rootstock for sweet orange, given rise to trees equal in vigour to those on the so-called vigorous rootstocks. *Mitha* (sweet lime), on the other hand, is quite vigorous and spreading on its own roots but, as a rootstock, it has dwarfed all the scion varieties budded on it in spite of its having made good union with the scion varieties. *Mokari* (citron), which is fairly dwarf on its own roots, has produced Sangtra plants of good vigour.

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# UTILIZATION OF VIRGINIA TOBACCO SEED IN THE MADRAS PROVINCE

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THERE is an area of about 3,00,000 acres under tobacco in the Madras province, of which nearly 1,50,000 acres is confined to the Guntur district, 50,000 acres to the Kistna and Godavari districts and the rest to the other parts of the province. Out of this, about 1,25,000 acres are grown with Virginia tobacco. Nearly 80 per cent of this area, in turn, is in the Guntur district and the rest in the Kistna and Godavari districts along the borders of the two rivers. The crop is allowed to flower and pod freely—unlike *natu*, the local cigar variety, which is invariably topped—in nearly 90 per cent of the Virginia tobacco-growing area to impart proper texture, body and colour to the leaf and make it fit for cigarette manufacture.

## *Seed, its oil content and general properties*

An average crop of Virginia tobacco yields about 150-200 lb. seed per acre. After the leaf picking is over and before the crop is pulled, sheep and goats are allowed to eat the pods freely when ripe, and they seem to relish it and do well. Majority of the seed pods are, however, burnt along with stalks which at present form the main fuel in use in all Virginia tobacco-growing areas. From preliminary trials it was found that the seed contained some oil and hence it was thought desirable to find some use for it. On extraction by the cold-drawn process in the country wooden mill (*chekku*) and by the hot-drawn process generally adopted for extracting castor oil, oil to an extent of 25-30 per cent could be got. The cold-drawn oil was thin, transparent, light yellow, pleasant smelling, agreeable to taste, and almost identical with first class gingelly oil. The hot-drawn oil was thin, slightly turbid yellow, pleasant smelling, and slightly bitter in taste. The seed and cake were readily eaten by sheep, goats and cattle. Viands made out of the cold-drawn oil obtained from well-washed seeds were not different from those made out of gingelly oil. Those prepared in the hot-drawn oil and in cold-drawn oil extracted from unwashed seeds were rather bitter to taste. The extraction of oil by the cold-drawn process as is done in the case of gingelly seeds will prove a good cottage industry in the Virginia tobacco areas, but there are not enough country wooden oil mills available in the areas for the purpose. Extraction by the hot-drawn process in the hand presses used for extracting castor oil is quite possible. Tobacco seeds, however, seem to

need a higher temperature and more pressure than castor seed for efficient extraction and to achieve this some structural changes in the oil presses now in use may be necessary. When the seed is pressed by this process, it should be crushed in flour mills before pressing for efficient extraction of oil.

*Chemical investigations of seed oil and cake*

Samples of oil and cake from the same batch were sent to several analysts in India and the results supplied by them are incorporated in Tables I-III. For purposes of comparison, the results obtained by investigators in other parts of the world on the subject are produced in Tables IV and V.

TABLE I

*Results of analysis of Guntur Virginia tobacco seed for food and mineral values*  
(By the Government Agricultural Chemist, Coimbatore)

Head of analysis	Per cent
<i>a. Food values—</i>	
Moisture . . . . .	6.05
Ash . . . . .	3.76
Crude proteins ( $N \times 6.25$ ) . . . . .	23.88
Ether extractives . . . . .	35.77
Crude fibre . . . . .	16.77
Carbohydrates (by difference) . . . . .	13.77
Total . . . . .	100.00
<i>b. Mineral values—</i>	
Insolubles . . . . .	0.31
Albuminoids (true proteins) . . . . .	22.80
Nicotine . . . . .	Absent*
<i>c. Mineral values—</i>	
Lime ( $CaO$ ) . . . . .	0.21
Potash ( $K_2O$ ) . . . . .	0.94
Phosphoric acid ( $P_2O_5$ ) . . . . .	1.09
Nitrogen (N) . . . . .	3.82

\* As observed by the Imperial Agricultural Chemist, New Delhi

The results obtained and the conclusions drawn therefrom were in general conformity with those obtained elsewhere and establish the fact that Guntur Virginia tobacco seed contains about 35-37 per cent of oil, is free from nicotine and can be used as an edible or semi-drying oil and for soap making or for illumination purposes. Its uses as an edible oil in Bulgaria are reported by Jamieson [1932] and as a semi-drying oil by Paris [1920] and Pyat-nitzkii [1929]. Samples of hard and soft soaps, prepared at the Kerala Soap Institute, Calicut indicate that the oil can be used for this purpose with success.

The cake contains about 30-35 per cent crude proteins, 29-34 per cent albuminoids, 16-17 per cent ether extractives, about 27 per cent carbohydrates, and is free from nicotine. It compares favourably in its food values with gingelly cake so extensively used for cattle feed in this province (Table VI) and cattle eat the cake freely. Feeding trials substituting tobacco seed cake for groundnut cake were conducted over a period of five weeks at the Agricultural Research Station, Guntur. The feeding did not bring about any adverse effects on the animals. They looked normal during the feeding trials and after. It is reported [Orlov, 1933] that the cake is extensively used for horse-feed. As the cake contains about 5 per cent nitrogen (N), 1.6 per cent phosphoric acid ( $P_2O_5$ ) and 1.15 per cent potash ( $K_2O$ ), it can be used as a good nitrogenous organic manure comparing favourably with an average sample of castor cake.

If facilities for the extraction or marketing of the oil do not exist, the seed can by itself be used as cattle food as it contains about 24 per cent crude proteins, 23 per cent albuminoids, 36 per cent ether extractives, 14 per cent carbohydrates, and is free from nicotine. As the seed has a thick seed-coat, it should be soaked in water for two days, washed well, ground into a paste like horse-gram and fed to cattle.

### *Further studies on seed oil*

Though the oil is fit for use for soap making and for illumination or edible purposes, its use as a semi-drying oil seems most advisable as the Madras province does not produce any other drying or semi-drying oil worth the name. For the supply of linseed oil which is the chief oil used for paints in the province, it depends entirely on northern India, and the typical drying oil—*tung* oil—is primarily imported from Burma and China.

The percentage composition of the different fatty acids in tobacco seed oil, linseed oil and *tung* oil are produced in Table VI, and some of their physical and chemical constants are given in Table VII. While *tung* oil stands unique in its composition and drying properties, linseed oil comes intermediate and tobacco-seed oil next in drying properties. The total absence of linolenic acid in tobacco-seed oil makes it dry somewhat slower, but its absence enables white paints mixed with tobacco-seed oil to continue to remain white, while they turn yellowish when mixed with linseed oil. While tobacco seed oil can be clarified to a practically colourless liquid, linseed oil cannot be so managed. These two characters of the oil make it eminently suited for the preparation and application of white paint and white enamels in preference to linseed oil.

TABLE II  
Some important physical and chemical constants of *Gunter Virginia tobacco seed oil*\*\*

Analysed by	Specific gravity at 30°C.		Refractive index at 40°C.		Acid value		Saponification No.		Iodine No.		Butyro-refractometer at 40°C.		Nicotine	
	C. D.	H. D.	C. D.	H. D.	C. D.	H. D.	C. D.	H. D.	C. D.	H. D.	C. D.	H. D.	C. D.	H. D.
1. Indian Institute of Science, Bangalore	...	...	1.46850	1.46850	0.800	0.595	190.4	190.5	124.4	124.9	...	...	Absent	Absent
2. Imperial Agricultural Chemist, New Delhi	...	...	...	...	...	...	...	...	137.1	138.7	...	...	...	...
3. Government Food Analyst, Gulbudy	...	...	...	...	0.55	0.39	...	...	142.0	142.0	64.7	65.3	...	...
4. Government Agricultural Chemist, Coimbatore	...	...	...	...	...	...	169.0	191.4	136.6	124.6	...	...	...	...
5. Superintendent, Kerala Soap Institute, Calicut	...	0.912	...	1.4725*	...	...	...	188.6	...	142.5	...	...	...	...
6. H. B. Technological Institute, Cawnpore	...	0.9153	...	1.4684	...	...	...	186.4	...	154.5	...	...	...	...

\*\*C. D. = Cold drawn ; H. D. = Hot drawn

\* At 15.5°C.  
+ At 30°C.

TABLE III

*Results of analysis (on dry basis) of Guntur Virginia tobacco seed cake for food and mineral values as against an average sample of gingelly cake*

Particulars	Tobacco seed cake, Analysed by						Gingelly cake, Analysed by Govt. Agrl. Chemist, Coimbatore
	Impl. Agrl. Chemist, New Delhi		Govt. Food Analyst, Guindy		Govt. Agrl. Chemist, Coimbatore		
	C. D.	H. D.	C. D.	H. D.	C. D.	H. D.	
<i>A. Food values</i> (per cent)							
Moisture . . . . .	5·87	5·22	..	..	..	..	..
Ash . . . . .	..	..	..	..	10·29	5·53	10·60
Crude proteins (N × 6·25)	30·75	35·81	..	..	30·58	34·13	37·90**
Ether extrac- tives	..	..	..	..	16·00	17·08	17·30
Crude fibre . . . . .	..	..	..	..	16·60	16·07	3·70
Carbohydrates (by difference).	..	..	..	..	26·53	27·19	30·50
Total . . . . .	..	..	..	..	100·00	100·00	100·00
True proteins or albuminoids	..	..	..	..	28·52	33·81	35·20
Oil . . . . .	..	..	11·2	14·5	..	..	..
Nicotine . . . . .	Absent	Absent					
<i>B. Mineral</i> <i>values (per cent)</i>							
Lime (CaO) . . . . .	..	..	..	..	On sample weight 0·65      0·22		
Potash (K <sub>2</sub> O) . . . . .	..	..	..	..	1·13	1·17	
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	..	..	2·5	2·6	1·85	1·41	
Nitrogen (N) . . . . .	4·92	5·73	4·6	5·2	4·89	5·46	

C. D. = Cold drawn ; H. D. = Hot drawn

\*\* Protein contents—

Gingelly cake from shelled seed . . . . .	33·13	} G. A. C., Coimbatore
Gingelly cake from un-shelled seed . . . . .	44·31	

TABLE IV

*Important characters of the tobacco seed oil as determined by investigators in other parts of the world*

Name of investigator	Ref. No.	Saponification value	Iodine value	Remarks
1. Ampola and Sourti [1904]	1	190·00	118·60	
2. Preissecker and Brezina [1919]	2	196·40	131·60	
3. Paris [1920]	3	194·60	135·46	Used as edible and semi-drying oil
4. Pyatnitzkii [1929]	4	200·70	135·34	Mixed with driers and pigments; rather slow-drying films were obtained
5. Morozov and Grashin [1930]	5	190·49	139·74	
6. Roberts and Schutte [1934]	7	182·00	152·00	Nicotine absent
7. Varga and Dedinszky [1934]	8	190·50	..	

TABLE V

*Results of analysis of tobacco seed cake for the food values (on dry basis) as determined by investigators in other parts of the world*

Particulars	Ref. 8	Ref. 10
	Per cent	Per cent
Moisture	3·38	8·66
Crude ash	4·97	14·43
Crude proteins	34·05	38·31
Crude fat	24·33	10·69
Crude fibre	23·64	17·97
Albumin (digestible)	..	36·37
Remarks.	No tobacco odour	Has been successfully used for horse feeding; nicotine absent

TABLE VI

*Percentage composition of different fatty acids in tobacco seed oil as compared to those in linseed oil and tung oil*

Name of acid	Tobacco seed oil			Linseed oil	Tung oil
	Ref. No. 4	Ref. No. 7	Ref. No. 11	(Ref. No. 12)	(Ref. No. 13)
	Per cent	Per cent	Per cent	Per cent	Per cent
Oleic. . . . .	21·70	16·20	26·37	5·00	10·00
Linoleic . . . . .	60·00	70·40	60·23	48 to 59	..
Linolenic . . . . .	..	..	..	21 to 32	..
Palmitic . . . . .	9·60	3·10	7·03	} 10·00	
Stearic . . . . .	..	4·80	3·04		
Elæostearic . . . . .	..	..	..		80·00
Glyceryl radicle . . . . .	..	..	..	4·60	
Unsapoifiable matter . . . . .	1·20	1·25	1·41	..	
Remarks . . . . .	Alcoloids and lino- lenic acid ab- sent				

### *Economic aspect*

At an estimate of 175 lb. seed per acre and 25 per cent of extractable oil in it, the province can supply about 8,800 tons of seed, or 2,200 tons of oil and 6,600 tons of cake per annum if the whole quantity of seed produced is gathered. If the oil is valued on par with the present price for linseed oil and cake on par with gingelly cake, the province can get annually enriched by 13 lakhs of rupees, if all the Virginia tobacco seed produced is utilized.\* This will incidentally make the province, at least partly, self-sufficient in its requirements for semi-drying oils.

Several paint firms have placed bulk indents for the supply of oil. Their quotations are, however, rather low, and the matter is under correspondence. A special grant of Rs. 500 has been sanctioned by the Madras Government to start business in this line.

\*An acre of Virginia tobacco contains about 5,000 plants and seed from about 10 plants will be more than sufficient to produce seedlings to plant an acre.

TABLE VII

*Some important physical and chemical constants of Guntur Virginia tobacco seed oil, linseed oil and tung oil, as noted in their raw form*

Particulars	Virginia tobacco seed oil (Guntur)	Linseed oil*	Tung oil*
Specific gravity at 15.5°C. . . . .	0.912 to 0.915	0.931 to 0.941	0.939 to 0.943
Refractive index at 20°C. . . . .	1.4684 to 1.4725	1.4742 to 1.4754	1.518 to 1.522
Acid number . . . . .	0.39 to 0.80	Up to 10	Up to 5
Saponification number . . . . .	186 to 191	189 to 196	189 to 195
Unsaponifiable matter . . . . .	1.2 to 1.41**	0.8 to 2.0	Up to 0.75
Iodine value . . . . .	124 to 155	170 to 185	155 to 167

\* Reference 13

\*\* Table IV

### *Future work*

Bulk samples of the oil have been sent to several leading firms manufacturing enamels, paints and varnishes, throughout India and their reports are awaited. Preliminary investigations made in the Sodhan Laboratories, Tenali, Guntur district, on the industrial utilization of the oil are sufficiently encouraging. Manurial trials with cake were arranged on all important crops grown under dry, irrigated and wet conditions during the year and they will be further elaborated during the next year. The technique of crushing is being studied for better extraction. If sufficiently encouraging reports are received from the different manufacturing firms, collection of seed and establishment of market for seed, oil and cake will be contemplated. In the meantime, necessary propaganda is being undertaken to utilize the seed as cattle food.

### CONCLUSIONS

Virginia tobacco seed extensively produced in the Guntur, Kistna and Godavari districts contains nearly 35-37 per cent of edible and semi-drying oil.

The oil can with advantage be used as semi-drying oil.

The cake can be fed to cattle or used as manure.

Till a market for seed, oil and cake is established, the seed can be fed to cattle.

#### ACKNOWLEDGEMENTS

Our thanks are due to the Imperial Agricultural Chemist, New Delhi ; Officer-in-Charge of Bio-Chemistry, Indian Institute of Science, Bangalore ; Principal, H. B. Technological Institute, Cawnpore ; Government Food Analyst, Guindy ; Director of Industries, Madras ; Superintendent, Kerala Soap Institute, Calicut ; Government Agricultural Chemist, Coimbatore and Manager, Sodhana Laboratories, Tenali, for the valuable information they have supplied.

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## REVIEW

### **Annual Review of Biochemical and Allied Research in India, Vol. XII, 1941**

Society of Biological Chemists, India, Bangalore : pp. 84 : price Rs. 3 or 6s.

**T**HIS review contains a faithful record of the activities of Indian workers in the field of biochemistry and allied subjects. Experts attached to representative institutions have been entrusted with the task of reviewing the work in branches.

Each of these reviews draws attention to the increased volume of work. Under soils and fertilizers, a short account is given of work done during the year on soils survey and classification, soil erosion, soil alkali and alkaline lands, physico-chemical properties of soils, methods of soil analyses, soil micro-organisms, nitrogen fixation and transformation in soils, soil conditions and crop production and manures and fertilizers. Under animal nutrition, research on the subjects of nutrition of livestock and dairy science have been reviewed. Work on vitamin-C contents of cow's milk and on various types of tinned milk, their food value, methods of manufacture, spoilage during storage have been included in this review. A review has been made of the quantitative and qualitative aspects of the existing food supply of the entire cattle population in India. In the same article, work on the digestibility and feeding value of oilseeds, on the effect of feeding berseem on growth and milk yield, on mineral requirements of cows and on nutritional requirements of chicken are summarized.

The publication of these annual reviews has been a regular feature of the activities of the Society of Biological Chemists for the last 12 years and for the valuable information it is supplying it can be regarded as an appendix to the world annual review of biochemistry.

## ERRATA

INDIAN JOURNAL OF AGRICULTURAL SCIENCE

*Vol. XII, Part III,*

Page 495, line 5, for 'velatile' read 'volatile'

Page 497, Fig. 2, for 'VI sample' read 'IV sample'

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*Vol. XII, Part V, October 1942*

Page 688, Table V (latter part), columns 1 and 5, first line, for 'W<sub>3</sub>' read 'W<sub>2</sub>'

Page 688, Table V (latter part), heading of column 3, for 'D<sub>3</sub>' read 'D<sub>2</sub>'

Page 707, line 9, for 'as' read 'at'

Page 708, heading of Table V, for 'or' read 'of'

Page 718, line 4, for 'hte' read 'the'

Page 746, line 5, for 'grow h' read 'growth'

Page 753, line 14, for 'fertilissimal' read 'fertilissima'

Page 753, line 16, for 'Basa' read 'Basal'

Page 753, last line, for 'ittle' read 'little'

Page 755, line 4, for '[Singh, 1939' read '[Singh, 1939]'

Page 755, line 10, for 'soil-' read 'soils'

Page 755, line 11, for 'questios' read 'question'

Page 755, line 12, for 'energyn' read 'energy -'

Page 755, line 18, for 'p ants' read 'plants'

Page 783, line 1, for 'thant wice' read 'than twice'

Page 788, line 4, for 'have)' read 'have'

Page 789, line 28, for 'clause-' read 'clauses'

## ORIGINAL ARTICLES

### THE INFLUENCE OF VARIATIONS IN THE INTERVAL BETWEEN CUTTINGS ON THE YIELD AND CHEMICAL COMPOSITION OF SOME PERENNIAL GRASSES IN THE PUNJAB

BY

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(Received for publication on 1 December 1941)

THE Chemical Section of the Punjab Agricultural Department has during recent years carried out extensive studies of the nutritive values of natural and cultivated fodders of the province. The results of some of these investigations spread over a period of six to seven years have already been published by Lander [1937] in a bulletin called *Indian Grazing Conditions and the Mineral Content of some Indian Fodders*. A second edition of this bulletin, brought up to date, is in the press.

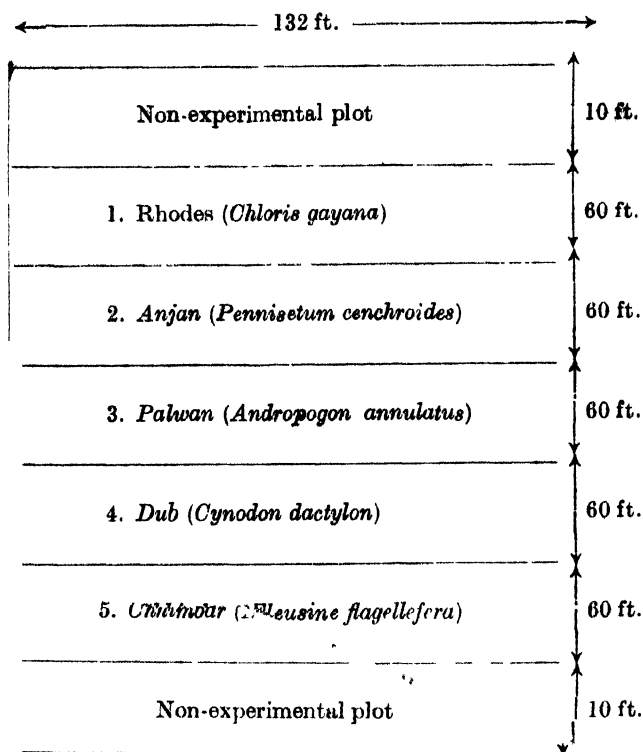
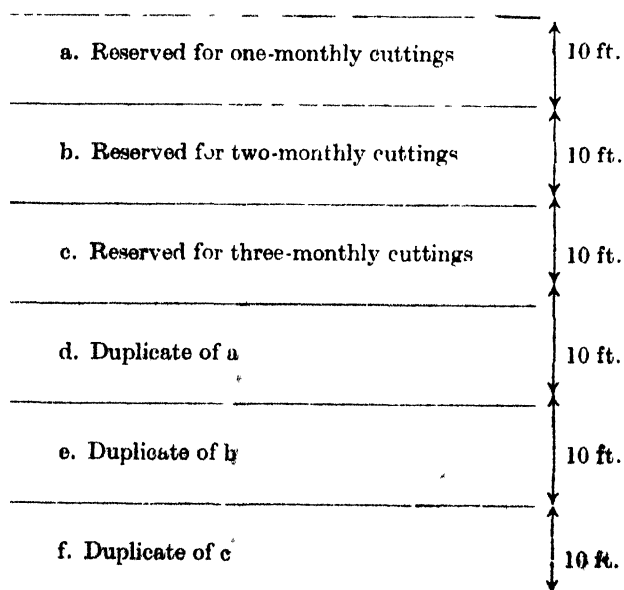
These investigations have to some extent followed, or been coincident with, work by the Fodder Specialist, in selecting various wild grasses of the province likely to prove valuable under special cultivated conditions.

A detailed investigation on the comparative yield and nutritive value of these specially selected grasses when cut at different stages of growth and development has been carried out in collaboration with the Fodder Specialist at the Botanical Sub-station at Sirsa in the Hissar district, a locality which has long been noted for the famous Hissar breed of cattle. In the bulletin referred to above, brief mention has been made of this work and some of the data available at the time of writing given. The investigation has now been completed and the results and conclusions arrived at are presented in this paper.

#### EXPERIMENTAL

The plans on the next page show the manner in which the experimental plot of land was divided amongst the various grasses investigated.

Plan A shows the lay-out of the plots under the five different grasses investigated, and plan B the manner in which each plot is further sub-divided into six sub-plots. It will be seen from plan B that from sub-plot a, a fresh growth of grass was obtained each month, while sub-plots b and c gave a number of cuttings two and three months old respectively. Sub-plots d, e and f are duplicates of a, b and c. Of these five grasses, *anjan*, *palwan*, *dub* and *chhimbar* are special selections made from the wild grasses of the province by the Fodder Specialist, whilst Rhodes grass is a promising exotic only recently introduced for trial at Sirsa. The land on which the grasses were grown was a loam soil of average fertility and all the grasses were planted in rows 2-3 ft. apart in the first week of March 1938. (For analyses of soil see Table IV). Meteorological data are presented in the appendix.

*Plan A**Plan B*

## SAMPLING AND ANALYSIS

In 1938 the grasses were sampled from June to November and not during the winter months, and as at that time there were no facilities on the spot for estimating moisture, data for total dry matter could not be obtained. Arrangements were made in due course to remedy this defect and in the following year complete data throughout the whole year were collected. Representative samples of grass from each cut were carefully dried at Sirsa for dry matter determinations and sent to Lyallpur for chemical analysis which included protein, ash, acid-soluble ash, lime, phosphoric acid and potash. The data of yield and chemical composition are given in Tables I-III.

## DISCUSSION OF RESULTS

## YIELD

Table I shows the total yield of freshly cut grasses and the various nutritive constituents obtained per acre during each year. The figures given represent the sum total of the individual yields corresponding to different cuttings obtained throughout the year in accordance with the rotations already mentioned.

## GREEN HERBAGE

It will be seen that *palwan* gave the highest yield of green grass and was followed by Rhodes and *anjan* whose yields were almost equal. *Dub* and *chhimbar* gave only about one-third the yield of *palwan* and do not compare with it or with Rhodes and *anjan*. If we consider the effect of the length of the interval between any two cuttings on the yield of grass, it will be seen that, except in the case of *dub*, the yield of green grass is greater the longer the interval between cuttings. This was especially noticeable in the case of *palwan*, Rhodes and *chhimbar*. These results are in agreement with those obtained by Paterson [1933 ; 1935] for some tropical grasses and Woodman *et al.* [1929] for pasture grasses in temperate regions.

## DRY MATTER

In computing the nutritive requirements of animals the usual procedure is to base estimates on the dry matter of the feed given and not on the total bulk of the green material. This is an important point to be borne in mind in view of the fact that the total yields of dry matter obtained are usually greater the longer the interval of time between any two cuttings. If we consider a single entire experimental period it will be seen from Tables I and II that there was a greater total quantity of grass obtained after longer intervals and also that the percentage of total dry matter was greater. As a general rule it may be said that in the case of these grasses the yield of dry matter is proportional to the yield of green grass.

## PROTEIN

The data for the two years given in this paper indicate a tendency for varieties which give the highest yields of dry matter to give also the highest

TABLE I

Yield of grass and its various constituents at different stages of maturity

Serial No.	Description	Yield in maunds per acre					Percentage variation from the monthly cutting						
		1939-40					Grass	Dry matter	Protein	CaO	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	
		Grass	Dry matter	Protein	CaO	P <sub>2</sub> O <sub>5</sub>							
1	Rhodes, one-monthly	143.7	45.28	3.263	0.400	0.332	0.818						
2	Rhodes, two-monthly	145.8	49.32	2.652	0.423	0.327	0.709	+1.46	-8.9	+10.7	-1.5	-13.3	
3	Rhodes, three-monthly	157.4	77.69	2.993	0.621	0.430	1.035	+9.56	-17.6	+55.7	+29.4	+26.5	
4	Anjan, one-monthly	134.4	41.44	3.311	0.339	0.336	1.407						
5	Anjan, two-monthly	147.1	45.35	2.581	0.381	0.340	1.346	+9.45	-9.4	+12.4	+1.2	-4.3	
6	Anjan, three-monthly	152.7	63.65	2.657	0.542	0.429	1.530	+13.62	-19.5	+59.9	+27.7	+8.7	
7	Palawan, one-monthly	167.5	62.12	4.798	0.398	0.251	1.250						
8	Palawan, two-monthly	192.3	77.57	3.923	0.441	0.230	1.316	+14.5	-24.9	+19.8	-13.2	+5.3	
9	Palawan, three-monthly	190.3	102.79	4.957	0.571	0.313	1.600	+13.6	+65.5	+43.5	+11.4	+23.0	
10	Dub, one-monthly	63.1	33.09	2.133	0.245	0.165	0.456						
11	Dub, two-monthly	59.6	30.30	1.355	0.255	0.138	0.364	-5.0	-3.4	-4.1	-16.4	-25.1	
12	Dub, three-monthly	61.0	33.44	1.697	0.317	0.171	0.438	-3.3	+10.2	+29.4	+3.6	-9.9	
13	Chimbar, one-monthly	28.5	16.27	1.411	0.131	0.100	0.252						
14	Chimbar, two-monthly	35.2	17.52	0.894	0.149	0.099	0.208	+23.5	+7.7	+13.7	-1.0	-17.5	
15	Chimbar, three-monthly	39.6	27.51	1.306	0.247	0.144	0.333	+39.0	+69.1	+88.6	+44.0	+32.2	

		1940-41											
		97.4	27.30	2.178	0.212	0.221	0.477						
1	Rhodes, one-monthly	.	.	.	.	.	.	.	.	.	.	.	.
2	Rhodes, two-monthly	128.1	47.79	2.556	0.340	0.331	0.714	+31.5	+75.1	+17.4	+60.4	+49.8	+49.7
3	Rhodes, three-monthly	143.9	59.39	2.336	0.407	0.381	0.705	+52.9	+117.6	-7.3	+92.0	+72.4	+47.8
4	Anjan, one-monthly	133.0	34.15	2.653	0.274	0.305	1.218	.	.	.	.	.	.
5	Anjan, two-monthly	155.9	47.91	2.762	0.382	0.337	1.420	-17.2	+40.3	+4.1	+67.5	+10.5	+16.6
6	Anjan, three-monthly	154.1	59.68	2.570	0.525	0.402	1.275	+15.9	+74.8	-3.1	+92.0	+31.3	+4.7
7	Palwan, one-monthly	129.1	45.08	2.810	0.312	0.217	0.838	.	.	.	.	.	.
8	Palwan, two-monthly	156.8	60.88	2.543	0.384	0.216	0.943	+21.5	+35.0	-9.3	+23.1	-0.5	+12.5
9	Palwan, three-monthly	208.4	94.88	2.626	0.503	0.255	1.085	+61.4	+110.5	-0.6	+80.5	+17.5	+23.5
10	Dub, one-monthly	22.0	10.93	0.653	0.091	0.061	0.159	.	.	.	.	.	.
11	Dub, two-monthly	23.1	12.25	0.625	0.102	0.061	0.147	+5.0	+12.1	-5.0	+12.1	0.0	-6.4
12	Dub, three-monthly	22.3	11.87	0.610	0.105	0.058	0.137	+1.4	+8.6	-7.3	+15.4	-5.0	-12.3
13	Chhimbar, one-monthly	13.3	5.88	0.419	0.049	0.042	0.077	.	.	.	.	.	.
14	Chhimbar, two-monthly	15.7	7.42	0.439	0.068	0.051	0.089	+18.0	+30.6	+4.8	+33.8	+21.4	+12.6
15	Chhimbar, three-monthly	17.9	9.40	0.492	0.083	0.065	0.106	+34.6	+65.5	+11.5	+69.4	+54.3	+37.7

TABLE II  
Average composition of grass at different stages of maturity

Serial No.	Description	Dry matter (per cent)	Percentage oven-dried material				P <sub>2</sub> O <sub>5</sub> /CaO equivalent CaO : 1	Percentage variation from the monthly cutting			
			Protein	CaO	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O		Protein	CaO	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O

1939-40											
1	Rhodes, one-monthly	31.5	8.00	0.88	0.73	1.81	0.93				
2	Rhodes, two-monthly	33.3	5.83	0.90	0.66	1.44	0.87	-32.8	+2.3	-9.6	-20.5
3	Rhodes, three-monthly	49.4	3.85	0.80	0.55	1.33	0.81	-51.9	-9.1	-24.7	-26.6
4	Arjua, one-monthly	30.9	7.99	0.82	0.81	3.39	1.17				
5	Arjua, two-monthly	30.8	5.89	0.84	0.75	2.97	1.06	-23.8	+2.4	-7.4	-12.4
6	Arjua, three-monthly	41.7	4.17	0.85	0.67	2.40	0.93	-47.8	+3.7	-17.3	-29.2
7	Palasa, one-monthly	37.1	7.72	0.64	0.45	2.01	0.83				
8	Palasa, two-monthly	40.3	5.06	0.57	0.30	1.70	0.82	-34.5	-10.9	-33.3	-15.4
9	Palasa, three-monthly	54.0	3.95	0.56	0.30	1.56	0.83	-43.8	-12.5	-33.3	-22.4
10	Dab, one-monthly	52.4	6.45	0.74	0.50	1.47	0.80				
11	Dab, two-monthly	50.8	4.57	0.78	0.46	1.20	0.70	-29.1	+5.4	-8.0	-18.4
12	Dab, three-monthly	63.0	4.41	0.82	0.45	1.14	0.65	-31.6	+10.8	-10.0	-22.5
13	Chikimbar, one-monthly	57.1	8.07	0.81	0.62	1.55	0.90				
14	Chikimbar, two-monthly	49.8	5.10	0.85	0.57	1.19	0.79	-41.2	+4.9	-8.1	-23.2
15	Chikimbar, three-monthly	69.5	4.75	0.90	0.52	1.21	0.63	-45.2	+11.1	-16.1	-21.9



TABLE III

*Yield and average composition of grasses at different stages in different seasons of the year*

Serial No.	Description	Dry matter (per cent)	Per cent oven-dried material				Yield of grass in md. per acre
			Protein	CaO	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	
Summer (April-September)							
1	Rhodes, one-monthly	32.1	7.66	0.86	0.71	1.85	111.2
2	Rhodes, two-monthly	33.2	5.15	0.84	0.62	1.48	110.8
3	Rhodes, three-monthly	53.6	3.67	0.77	0.52	1.30	119.5
4	Anjan, one-monthly	31.6	7.65	0.81	0.76	3.40	97.4
5	Anjan, two-monthly	31.3	5.30	0.82	0.71	3.05	111.9
6	Anjan, three-monthly	45.2	3.79	0.83	0.66	2.50	112.6
7	Palwan, one-monthly	38.0	7.60	0.64	0.44	2.04	121.0
8	Palwan, two-monthly	41.0	5.02	0.57	0.28	1.68	142.2
9	Palwan, three-monthly	57.4	3.96	0.54	0.30	1.58	145.2
10	Dub, one-monthly	53.9	6.48	0.73	0.48	1.48	49.9
11	Dub, two-monthly	51.7	4.41	0.76	0.45	1.21	52.7
12	Dub, three-monthly	64.4	4.27	0.81	0.44	1.14	55.1
13	Chhimbar, one-monthly	59.7	8.69	0.78	0.60	1.57	24.4
14	Chhimbar, two-monthly	50.1	4.96	0.84	0.56	1.19	31.5
15	Chhimbar, three-monthly	72.0	4.65	0.89	0.51	1.21	34.5
Winter (October-March)							
1	Rhodes, one-monthly	29.5	9.28	0.97	0.84	1.64	32.5
2	Rhodes, two-monthly	36.0	6.04	1.06	0.78	1.32	35.0
3	Rhodes, three-monthly	36.0	4.70	0.94	0.73	1.50	37.9
4	Anjan, one-monthly	28.8	8.96	0.85	0.95	3.37	37.0
5	Anjan, two-monthly	29.4	7.03	0.91	0.87	2.70	35.2
6	Anjan, three-monthly	51.6	5.70	0.95	0.74	2.02	40.1
7	Palwan, one-monthly	34.8	8.07	0.66	0.50	1.95	46.5
8	Palwan, two-monthly	38.6	5.16	0.55	0.35	1.75	50.1
9	Palwan, three-monthly	43.0	3.90	0.65	0.33	1.46	45.1
10	Dub, one-monthly	46.9	6.32	0.79	0.57	1.42	13.2
11	Dub, two-monthly	44.5	5.99	0.91	0.49	1.14	6.9
12	Dub, three-monthly	50.5	6.08	1.04	0.47	1.07	5.9
13	Chhimbar, one-monthly	41.2	8.52	1.01	0.71	1.36	4.1
14	Chhimbar, two-monthly	47.0	6.38	0.98	0.63	1.21	3.7
15	Chhimbar, three-monthly	52.5	5.63	0.97	0.63	1.19	5.1

TABLE III--*contd*

Serial No.	Description	Dry matter (per cent)	Per cent oven-dried material				Yield of grass in md. per acre
			Protein	CaO	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	
Summer (April-September)							
1	Rhodes, one-monthly . . .	27.5	7.50	0.74	0.76	1.78	78.2
2	Rhodes, two-monthly . . .	38.8	4.89	0.66	0.63	1.50	93.7
3	Rhodes, three-monthly . . .	41.6	3.66	0.60	0.59	1.13	110.2
4	Anjan, one-monthly . . .	24.9	7.58	0.74	0.84	3.70	106.1
5	Anjan, two-monthly . . .	30.7	5.45	0.70	0.61	3.05	119.4
6	Anjan, three-monthly . . .	40.6	4.09	0.79	0.62	2.10	115.3
7	Palwan, one-monthly . . .	34.6	6.24	0.66	0.47	1.95	101.1
8	Palwan, two-monthly . . .	38.8	4.02	0.61	0.34	1.60	125.8
9	Palwan, three-monthly . . .	47.0	2.58	0.55	0.25	1.04	162.8
10	Dub, one-monthly . . .	49.4	5.93	0.80	0.56	1.50	17.4
11	Dub, two-monthly . . .	52.8	4.95	0.78	0.49	1.24	18.8
12	Dub, three-monthly . . .	52.2	4.95	0.83	0.48	1.15	17.8
13	Chhimbar, one-monthly . . .	42.4	7.22	0.81	0.71	1.38	11.3
14	Chhimbar, two-monthly . . .	47.8	5.73	0.86	0.67	1.23	13.1
15	Chhimbar, three-monthly . . .	53.4	5.03	0.84	0.67	1.13	14.8
Winter (October-March)							
1	Rhodes, one-monthly . . .	30.3	9.74	0.91	0.98	1.62	19.2
2	Rhodes, two-monthly . . .	33.3	6.81	0.89	0.89	1.48	34.4
3	Rhodes, three-monthly . . .	35.1	4.86	0.98	0.81	1.39	38.7
4	Anjan, one-monthly . . .	28.9	8.42	1.03	1.06	3.11	26.9
5	Anjan, two-monthly . . .	30.7	6.79	1.12	1.00	2.67	36.5
6	Anjan, three-monthly . . .	33.0	5.11	1.20	0.86	2.30	38.8
7	Palwan, one-monthly . . .	35.8	6.21	0.80	0.51	1.53	28.0
8	Palwan, two-monthly . . .	38.8	4.87	0.73	0.40	1.35	31.0
9	Palwan, three-monthly . . .	40.4	3.52	0.77	0.35	1.30	45.6
10	Dub, one-monthly . . .	50.7	6.35	0.94	0.56	1.20	4.6
11	Dub, two-monthly . . .	53.9	5.73	1.08	0.52	1.03	4.3
12	Dub, three-monthly . . .	57.3	5.81	1.08	0.50	1.16	4.5
13	Chhimbar, one-monthly . . .	44.5	8.20	1.12	0.90	1.24	2.0
14	Chhimbar, two-monthly . . .	44.6	6.90	1.21	0.78	1.03	2.6
15	Chhimbar, three-monthly . . .	48.4	6.34	1.13	0.80	1.13	3.1

yields of protein, but the data for the two successive years are not consistent. In 1939-40, although the total monthly cuttings of all the grasses under trial yielded less dry matter than the two and the three-monthly cuttings, the higher protein content of the young grass (Table II) more than counterbalanced the effect of the lower yield, with the result that the monthly cuttings gave a greater protein yield in that year. It was further found that three-monthly cuttings gave a greater sum total of protein than the two-monthly cuttings. This finding, however, did not hold strictly true in 1940-41 when the yield of protein in the monthly cuttings was not consistently greater in all cases. This may be explained by the fact that in the case of some varieties the percentage increase of green grass and dry matter in the case of two and three-monthly cuttings was much greater in 1940-41 than in 1939-40, and consequently the higher protein content of the individual monthly cuttings did not match the high protein resulting from the greater yield of dry matter in 1940-41.

#### LIME, PHOSPHORIC ACID AND POTASH

It will be seen that as the interval between any two cuttings increases the sum total yield of lime is greater, but the variations in the total yields of phosphoric acid and potash were not regular. The data obtained therefore do not yield any conclusive evidence that different cutting rotations have any pronounced effect on the total yield of either of these two constituents.

#### CHEMICAL COMPOSITION

The results of the mean chemical composition of the various grasses cut at different intervals of time are given in Table II, in the construction of which the original data and the absolute amounts of the various constituents obtained from different plots for an entire year have been employed. The figures for mean composition given in Table II have been arrived at by dividing the total amounts of the various constituents by the total amounts of dry matter and expressing the former as percentages of the latter. It will be seen that there are definite variations in the percentages of some of the chemical constituents in the grasses, e.g. *anjan* is particularly rich in potash, while *palwan* is very poor in both lime and phosphoric acid. With these exceptions, however, there appear to be no major differences in the chemical composition of any of these grasses from similar cuttings. In regard to differences in chemical composition when grasses are cut after varying intervals of time, it will be seen that :—

1. There was a progressive increase in the percentage of dry matter as the interval between any two cuttings increased.
2. The percentage of protein, phosphoric acid and potash decreased with increasing intervals of time between cuttings, the decrease being more marked in the case of protein.
3. The percentage content of lime appears to be independent of time intervals but there is sometimes a slight fall in the lime content and at others a slight rise.

#### RATIO OF LIME AND PHOSPHORIC ACID

While the functions of both lime and phosphoric acid in fodders has been recognized for long, the far greater importance of the ratio of the two

has only been brought into prominence in recent years. Crowther [1939] suggests that this ratio should be 1 : 1.5 expressed as equivalents of CaO and  $P_2O_5$ , because this is the ratio in which these two minerals exist in milk. It has accordingly been calculated and given in Table II as phosphorus-calcium equivalents. It will be seen from these figures that although none corresponds with the standard figures mentioned above, the monthly cuttings of *anjan* and Rhodes in the 1940-41 season approached nearest to the standard. It will be further noted that as the intervals between the cuttings increased, the ratios tended to deviate more from the standard, because, as already pointed out, as the interval between cuttings increases the phosphoric acid content decreases but the lime remains more or less constant, thus introducing a deviation in the ratio of these constituents. This is a very important point because although, as we have seen earlier, longer interval between cuttings increases the total yield of dry matter it nevertheless disturbs the very important lime-phosphorus ratio.

#### EFFECTS OF SEASON

The effect of variations in the season on chemical composition is clearly brought out in Table III. The data from two arbitrary periods, viz. summer, from April to September, and winter, from October to March, have been separated and the mean composition of the grasses for the two periods calculated on the same lines as were adopted in the construction of Table II.

It will be seen that the total yield of fresh grasses obtained in the winter period is much less than that obtained during the summer period, the ratio being from one-third to one-fourth. This would naturally be expected as growth is much more luxuriant during the high temperature and monsoon rains of summer than in winter. Woodman and Oosthuizen [1934] have reported on the chemical composition of winter and summer pasture grasses in England, where the winters and summers are not comparable with Indian winters and summers. It would be more strictly accurate to compare the English summer with the Punjab winter from a climatic point of view. These observers found in the case of English pasture grasses that the summer crop is richer in protein, phosphoric acid and lime than the corresponding winter crop. Data for the Punjab show that although the yield of grass is less during winter, it is nevertheless richer in the above-mentioned constituents. During summer the high metabolic activity of the plant material is directed more to the formation of carbohydrates than protein material and the absorption of calcium and phosphorus by the roots. This naturally results in a picture of the chemical composition of the winter and summer grasses, the reverse of that found in temperate climates. An interesting point which is worth attention is the fact that although the Punjab winter grasses of from one to two months old were richer in nitrogen, calcium and phosphorus than summer grass, they were poorer in their potash content. In the case of grass which was three months old, however, the content of potash in the winter grass was greater than that in the summer grass of similar age. Some explanation may be found for the high potash content of summer grasses in the necessity of the plant to meet particular seasonal requirements. During summer photosynthetic activity is greater than in winter and as potash is

TABLE IV  
*Soil analyses*  
(Plots 1—5, Plan A)

Serial No.	Description	10 per cent water extract					Mechanical analyses						
		Total solids	Na <sub>2</sub> CO <sub>3</sub>	NaHCO <sub>3</sub>	NaCl	Na <sub>2</sub> SO <sub>4</sub>	Ca	Clay 0.0-0.002 mm.	Silt 0.002-0.02 mm.	Fine sand 0.02-0.2 mm.	Sand 0.2-2.0 mm.	Gravel above 2 mm.	Calcium carbonate
1	1st plot, 1st ft.	0.120	Nil	0.076	0.027	0.017	0.016	15.24	13.44	70.09	Nil	0.56	1.23
2	" " 2nd "	0.132	"	0.076	0.028	0.010	0.017	18.38	17.10	63.62	"	0.50	0.90
3	2nd " 1st "	0.110	"	0.076	0.025	0.007	0.016	15.20	16.52	66.63	"	0.17	1.65
4	" " 2nd "	0.140	"	0.076	0.039	0.007	0.021	22.52	21.44	54.14	"	1.04	1.90
5	3rd " 1st "	0.124	"	0.076	0.032	0.009	0.018	16.16	13.36	68.43	"	3.69	2.05
6	" " 2nd "	0.126	"	0.076	0.035	0.006	0.034	19.92	20.34	55.26	"	4.34	4.48
7	4th " 1st "	0.159	Traces	0.076	0.062	0.011	0.019	16.18	10.18	71.91	"	0.37	1.73
8	" " 2nd "	0.176	Nil	0.076	0.076	0.013	0.024	20.62	18.34	55.41	"	0.92	6.23
9	5th " Born A, 1st ft.	0.148	"	0.076	0.056	0.018	0.018	15.24	10.80	72.58	"	Nil	1.38
10	" " " 2nd "	0.164	"	0.076	0.060	0.010	0.018	18.28	14.48	62.79	"	4.96	4.45

considered to play an important part in regulating this particular activity, the content of potash in summer would naturally be expected to be greater compared with other food constituents.

#### SUMMARY

The paper describes the effects of intervals of cutting and of season on the yield and chemical composition of some important perennial grasses of the Punjab.

Longer intervals between two cuttings gave greater yields of both green herbage and dry matter.

A progressive fall of protein, phosphoric acid and potash contents of the dry matter in the grass occurs with increasing intervals of time between cuttings.

Varying intervals of time between cuttings do not affect the lime content of grasses.

Grasses yield much more dry matter in summer than in winter, but the herbage obtained in summer was poorer in protein, calcium and phosphorus than the corresponding herbage obtained in winter.

Although young herbage obtained in summer was poorer in its calcium and phosphoric acid contents, it was richer in potash than the winter herbage.

From the point of view of the yield of dry matter, the grasses investigated may be arranged in the order: *palwan*, *anjan* (and *Rhodes*), *dub* and *chhimbar*: with respect to the calcium and phosphorous contents, however, *palwan* comes last, the rest not showing any significant differences.

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APPENDIX  
*Meteorological data for Sirsa*

Month	1939			1940			1941			Normal (average) rainfall (in.)
	Rainfall (in.)	Mean maximum temperature (°F.)	Mean minimum temperature (°F.)	Rainfall (in.)	Mean maximum temperature (°F.)	Mean minimum temperature (°F.)	Rainfall (in.)	Mean maximum temperature (°F.)	Mean minimum temperature (°F.)	
January . . . . .				1.29	68.59	36.90	2.40	69.06	46.79	0.60
February . . . . .				0.29	72.0	39.71	0.15	81.91	51.91	0.96
March . . . . .	1.02	74.95	44.62	0.19	83.2	51.22	...	99.5	62.93	3.42
April . . . . .	...	92.16	58.93	0.09	99.9	64.69				0.28
May . . . . .	...	107.08	77.36	0.02	112.6	77.41				0.53
June . . . . .	2.59	100.94	76.50	3.15	109.9	85.11				1.66
July . . . . .	0.54	98.24	84.88	1.94	104.84	86.94				3.57
August . . . . .	...	102.53	78.69	3.94	93.54	83.26				3.07
September . . . . .	...	101.9	74.13	0.35	93.35	83.33				2.41
October . . . . .	...	95.74	61.74	...	89.25	73.66				0.25
November . . . . .	...	83.68	44.53	...	91.17	53.28				0.06
December . . . . .	...	75.44	35.19	...	77.66	46.51				0.34

# SOME OBSERVATIONS ON THE GROWTH OF THE COCONUT FRUIT WITH SPECIAL REFERENCE TO SOME OF THE CHANGES UNDERGONE BY THE FIBROUS CONSTITUENT OF ITS MESOCARP

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(With two text-figures)

**I**N view of the facts that the coconut, unlike many other fruits, is composed of several physically and chemically distinct components, that each of these components has unique economic value, and that the fruit takes normally more than a year to ripen and fall down, it was thought expedient to trace the growth of the fruit with particular reference to the changes occurring in its several components. It is true that fairly detailed descriptions of the growth of the fruit are found in such classical treatises on the coconut palm as those of Copeland [1931], Sampson [1923] and others, but those studies have not been of the strictly scientific type involving due insistence on all possible measurements and analyses. The present study is an attempt to exclude that serious defect and indicate a possible line of successful attack of several problems relating to the growth of this important agricultural product.

## MATERIAL AND METHODS

It is a matter of familiar observation that coconuts belonging to the same bunch make the greatest approach to uniformity. Nuts of the same bunch are very nearly of the same age. Further, equidistribution of nourishment is ensured among these nuts, and they undergo identical vicissitudes of atmospheric and soil conditions. The approximate uniformity of the nuts in regard to shape, size and weight is to be attributed to these causes.

There is a general rule that the spacing in time of any two consecutive bunches on the palm is almost a constant, viz. a month. Whatever may be the value of this rule in general practice, it is undependable for rigorous scientific study. Conclusions drawn, therefore, from the measurements and analyses of nuts picked from different bunches of the tree at the same time are lacking in exactitude. Moreover, it has been observed that the coconut fruit is extremely sensitive to weather conditions, and inasmuch as different bunches have experienced different states of the weather, new factors creep in and vitiate the records set up by the mere passage of time.

In view of these factors, the method was adopted of keeping under observation the same bunch for a period of about 12 months during which period the nuts constituting the bunch grew from infancy and reached maximum

maturity on tree. The bunch with the largest formation of nuts was selected and on a specific date of each successive month a man was sent up the tree and a nut detached from the bunch. This nut was then measured and analysed in accordance with the scheme embodied in the tables. For the purpose of confirmation of results and of conclusions drawn therefrom, the experiment was conducted on three trees, and two bunches were marked out on each tree.

The nut was weighed immediately after picking, and its volume determined in a specially constructed over-flow jar, a lead sinker of known volume being used whenever necessary. The kernel was scooped or cut out of the shell as required and weighed in the raw state. The husk was torn to pieces and boiled in about 2-3 litres of water with 5-10 gm. of sodium sulphite, for a couple of hours, when it softened to such an extent as to render possible the easy separation of the fibrous from the non-fibrous matter by simple mechanical means. After separation and thorough washing, each component was air-dried and weighed.

Before determining the specific gravity of the fibre and subjecting it to chemical analyses, a further purification was effected by boiling the finely divided fibre with 20 per cent acetic acid for half an hour, followed by repeated washings with distilled water, this procedure having been found suitable in previous work of the author [Menon, 1935]. The fibre was then dried, and after air-conditioning stored in a stoppered bottle.

Determination of the specific gravity of the various samples of fibre presented difficulty at first. Using the well-known method of the specific gravity bottle, it was found that there was no early limit to the time for which bubbles of air escaped from the immersed fibre. Consequently, values obtained by this method on the same sample disagreed profoundly. Complete satisfaction was secured, however, when the fibre was first boiled in a beaker with distilled water for about 15 minutes, when all the capillary air was expelled and the particles settled down. The beaker with contents was then rapidly cooled, and the fibre transferred into the specific gravity bottle by means of balance forceps. The bottle was then filled with water, stopper replaced, and weighings made as usual. The fibre was carefully shaken out from the bottle into a filter cone, which was later dried in the oven. The dry fibre was next transferred into a weighing tube, which was then introduced into a special drying apparatus by the aid of which its bone-dry weight was determined. This value represented the weight of fibre in the specific gravity calculation.

Lignin and furfural values recorded in Tables IV and V are based on the bone-dry fibre, and were estimated by the familiar 72 per cent sulphuric acid and Tollen's methods respectively.

### DISCUSSION

Figs. 1 and 2 throw light on the value of the method adopted for tracing the growth of the coconut fruit.

When measured in C. G. S. units, the weight and volume of the fruit are very nearly equal for the first six months of growth, which in other words means that the specific gravity of the fruit taken as a whole is almost the same as that of water. After this period, the weight of the nut rapidly decline

until it becomes less than half the maximum value it had reached. This decline is in spite of the fact that the kernel goes on increasing in weight and the diminution in weight of the nut water is only a negligible fraction of the total loss in weight of the fruit. The obvious explanation is that the husk which is heavily soaked in water during the earlier stages of growth begins rapidly drying up even while the kernel is in the process of formation and undergoing active synthetic reactions. The volume of the fruit remains, however, constant but for the slight diminution that takes place owing to shrinkage as the fruit dries up.

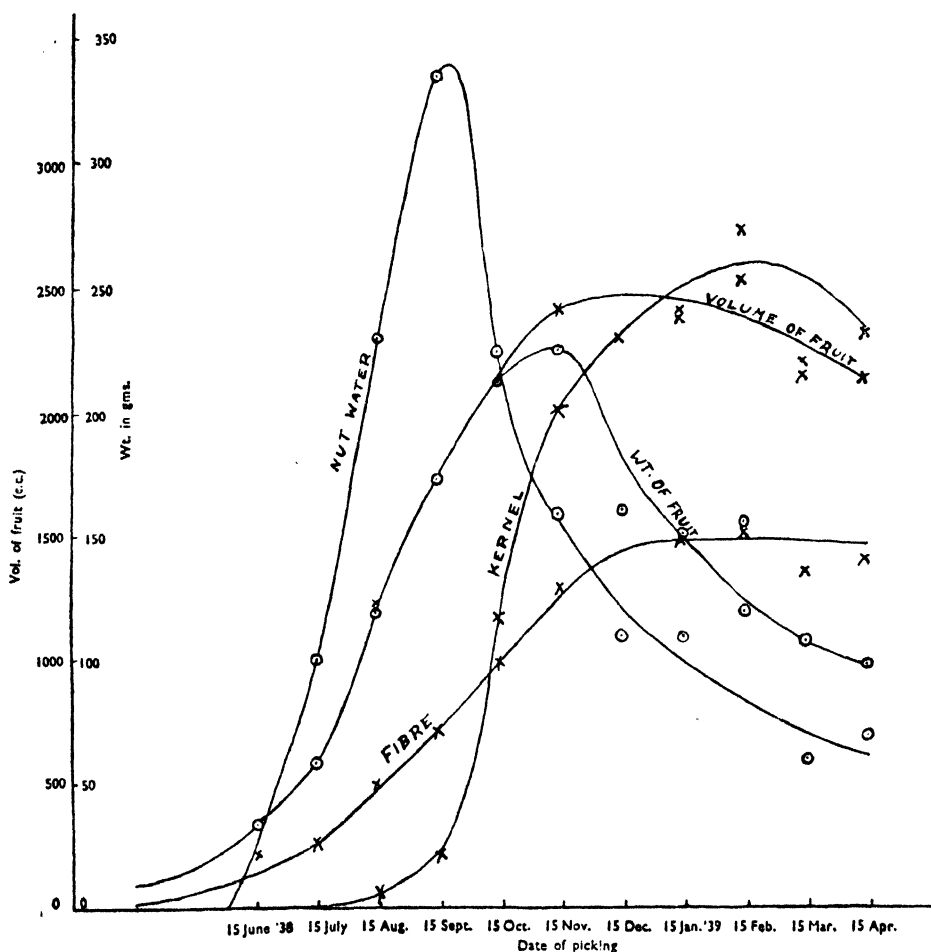


FIG. 1. Growth of fruit (Tree No. 171, Bunch No. 1)

TABLE I

*Tree No. 171*

Date of picking	No. of nuts in bunch	Weight of fruit (gm.)	Vol. of fruit (c.c.)	Vol. of nut water (c.c.)	Weight of kernel (gm.)	Weight of fibre (gm.)	Weight of pith (gm.)
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*Bunch No. 1*

15 June 1938	14	..	348	..	0	23	5
15 July „ .	12	581	585	100	0	26	14
15 Aug. „ .	11	1193	1230	230	7	50	31
15 Sept. „ .	10	1720	1710	335	22	72	44
15 Oct. „ .	9	2122	2150	225	117	98	46
15 Nov. „ .	8	2250	2420	160	201	129	75
15 Dec. „ .	7	1614	2300	110	199	131	71
15 Jan. 1939	5	1515	2380	110	241	148	84
15 Feb. „ .	4	1563	2530	120	272	151	103
15 March „ .	3	1086	2145	60	219	134	92
15 Apr. „ .	0*	984	2135	70	231	140	103

*Bunch No. 2*

15 June 1938	9	..	1048	..	0	59	9
15 July „ .	8	1699	1690	325	9	71	39
15 Aug. „ .	7	2385	2450	293	120	98	59
15 Sept. „ .	6	2457	2582	210	187	126	82
15 Oct. „ .	5	2398	2680	195	246	163	93
15 Nov. „ .	4	2063	2915	140	299	161	128
15 Dec. „ .	3	1429	2590	95	262	149	100
15 Jan. 1939	2	1510	2760	125	284	172	113
15 Feb. „ .	1	1263	2550	83	267	156	106

\*Both the remaining nuts grew dead ripe and fell down before date ; one of them was analysed and recorded as above

TABLE II

*Tree No. 152*

Date of picking	No. of nuts in bunch	Weight of fruit (gm.)	Vol. of fruit (c.c.)	Vol. of nut water (c.c.)	Weight of kernel (gm.)	Weight of fibre (gm.)	Weight of pith (gm.)
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*Bunch No. 1*

15 June 1938	12	..	623	..	0	35	11
15 July ..	10	1122	1090	250	0	51	29
15 Aug. ..	9	1600	1600	240	80	70	48
15 Sept. ..	8	1567	1560	145	162	83	53
15 Oct. ..	7	1625	1670	..	206	89	47
15 Nov. ..	6	1480	1610	85	204	99	76
15 Dec. ..	5	992	1525	40	211	96	52
15 Jan. 1939	4	895	1440	25	207	103	59
15 Feb. ..	3	864	1585	60	187	106	68
15 March ..	1	742	1640	30	222	118	83

*Bunch No. 2*

15 June 1938	12	..	316	..	0	21	7
15 July ..	11	584	540	115	0	29	17
15 Aug. ..	10	1129	1140	197	..	53	30
15 Sept. ..	9	1367	1350	170	91	73	45
15 Oct. ..	8	1562	1580	110	153	81	41
15 Nov. ..	7	1621	1660	105	174	99	79
15 Dec. ..	6	1245	1730	55	186	103	57
15 Jan. 1939	5	919	1520	55	172	100	59
15 Feb. ..	4	931	1740	50	252	115	72
15 March ..	3	765	1490	40	185	109	71

The two remaining nuts fell before 15th April 1939, after growing dead ripe

TABLE III  
Tree No. 145

Date of picking	No. of nuts in bunch	Weight of fruit (gm.)	Vol. of fruit (c.c.)	Vol. of nut water (c.c.)	Weight of kernel (gm.)	Weight of fibre (gm.)	Weight of pith (gm.)
<i>Bunch No. 1</i>							
15 June 1938 .	8	..	238	..	0	..	..
15 July „ .	7	604	590	135	0	28	15
15 Aug. „ .	6	1400	1430	297	0	60	45
15 Sept. „ .	5	1822	1860	335	34	89	53
15 Oct. „ .	4	1942	1960	255	91	100	66
15 Nov. „ .	3	1917	2035	180	143	110	107
15 Dec. „ .	2	1797	2240	175	221	133	88
15 Jan. 1939 .	1	1522	2185	155	263	137	92
<i>Bunch No. 2</i>							
15 June 1938 .	8	..	138	..	0	9.4	1.5
15 July „ .	7	308	340	65	0	18.5	11
15 Aug. „ .	6	912	897	207	0	41	27
15 Sept. „ .	5	1659	1700	345	6	82	52
15 Oct. „ .	3	1823	1830	300	57	97	57
15 Nov. „ .	2	1783	1845	180	139	104	87
15 Dec. „ .	1	1907	2330	205	209	129	83

This branching of the weight-volume curve signals the beginning of a new series of chemical changes within the husk. It will be observed in Figs. 1 and 2 that the increase in weight of the fibre is almost uniform during the early period of growth, but the rate of growth declines after the weight of the fruit has begun to diminish. The reason for this may be that the formation of the non-lignin constituents of the husk comes to an end as soon as the husk begins to dry up, and that thereafter the increase in weight of the fibre content is only due to the lignification of the fibre, which proceeds more rapidly after the husk has begun to dry up. There is reason to suppose that the type of lignification that takes place after the drying up of the husk has commenced is significantly different from the type that precedes it. In the former, it is more a case of deposition of phenolic compounds on the cellulosic framework of the fibre than

a case of chemical linkage of the ring compounds with the chain-like structure of the cellulose. In this connection the author's conclusions based on the study of the nature of the lignin complex of coir fibre [Menon, 1936] may appear meaningful. Table IV shows that the lignin value of the fibre goes on steadily increasing practically to the very last stages of growth of the fruit. This is in agreement with a previous observation of the writer [Menon, 1935] that the methoxyl value of coir fibre increases with the growth. Table V shows the quantity of non-lignin present in the fibrous matter of the fruit at the various stages of growth. Up to November 1938, the non-lignin content steadily increased, but the figures obtained for the next five months do not indicate, making due allowance for individual variation of nuts, that it increased subsequently. The practical significance of this observation is considerable. It proves that in picking the nuts before they are fully ripe, not only no loss in yield of fibre results unlike in the case of copra, but there is the striking advantage of obtaining a cleaner and whiter fibre that registers a higher non-lignin value and a lower lignin value. The advantage regarding colour and gloss is vital. The discoloration that the fibre is subjected to as the drying of the husk becomes intense and the extraneous tannin matter of the husk deposits itself on the fibres, is permanent and ineliminable; it is responsible for the very low prices obtained for such fibres in the market. The practice adopted, therefore, in Malabar of picking the nuts while they are still green, is scientifically justified from the view-point of coir production.

TABLE IV

*Analysis of coir fibre extracted from nuts of varying growth*

(Palm No. 171, bunch No. 1)

Date of picking	Age in months*	Moisture (per cent)	Specific gravity	Lignin (per cent)	Furfural (per cent)
15 June 1938 . . .	X	10.0	1.52	28.0	16.5
15 July „ . . .	X+1	8.9	1.52	27.4	16.0
15 Aug. „ . . .	X+2	10.0	1.50	31.1	15.5
15 Sept. „ . . .	X+3	9.3	1.50	32.1	..
15 Oct. „ . . .	X+4	10.0	1.50	34.1	..
15 Nov. „ . . .	X+5	10.3	1.51	34.4	14.3
15 Dec. „ . . .	X+6	9.7	1.50	34.3	..
15 Jan. 1939 . . .	X+7	..	1.49	35.5	..
15 Feb. „ . . .	X+8	9.6	1.48	36.4	14.4
15 March „ . . .	X+9	11.3	1.48	36.0	..
15 Apr. „ . . .	X+10	..	1.49	37.2	..

\*The value of X, as read from the graphs, is about 1 1/2—2 1/2 months

**TABLE V**  
*Variation of the non-lignin content of coir fibre with growth*

Date of picking	Weight of fibre gm. (W)	Lignin per cent (x)	Non-lignin per cent (100-x)	Weight of non-lignin in fibre $\frac{W(100-x)}{100}$ gr
15 June 1938 . . . . .	23	28.0	72.0	16.6
15 July „ . . . . .	26	27.4	72.6	18.9
15 Aug. „ . . . . .	50	31.1	68.9	34.5
15 Sept. „ . . . . .	72	32.1	67.9	48.9
15 Oct. „ . . . . .	98	34.1	65.9	64.6
15 Nov. „ . . . . .	129	34.4	65.6	84.6
15 Dec. „ . . . . .	131	34.3	65.7	86.1
15 Jan. 1939 . . . . .	148	35.5	64.5	95.5
15 Feb. „ . . . . .	151	36.4	63.6	96.1
15 March „ . . . . .	134	36.0	64.0	85.8
15 Apr. „ . . . . .	140	37.2	62.8	87.9

Another remarkable fact revealed by Table IV is that the specific gravity of the fibre during its growth undergoes a little alteration, but this alteration is surprisingly on the negative side. This may be of great theoretical significance, inasmuch as the process of increasing lignification is not only not attended by any increase in specific gravity of the lignified material, but attended by a perceptible diminution of the same.

The furfural value of the fibre too diminishes by slight degrees during its growth. Making allowance for the increasing proportion of lignin in the fibre, this indicates that the cellulosic portion of coir fibre is very rich in furfural-yielding substances from the very beginning. The proportion of such substances in the cellulose remains practically undiminished to the very end.

One fact remains to be mentioned regarding the husk of the coconut. The non-fibrous constituent of the husk, as has already been stated, was separated from the fibres by preliminary boiling with dilute sodium sulphite solution. The reaction that takes place is not quite clear, but it is found to be very effective in treating coconut husk during all stages of growth. The non-fibrous matter isolated from the immature nuts was not a corky powder as is commonly obtained from mature coconuts but a fine sticky paste which on drying assumed a leathery consistency. This paste was capable of binding the fibres into a tough mat. Elaborate experiments were recently conducted on this subject,

followed by large-scale trials at the Forest Research Institute, Dehra Dun, and a patented process has been perfected for the manufacture of a variety of useful articles, now styled as 'Menonite' products, from the entire husk of immature coconuts naturally falling from the tree and obtained as an agricultural waste in coconut plantations.

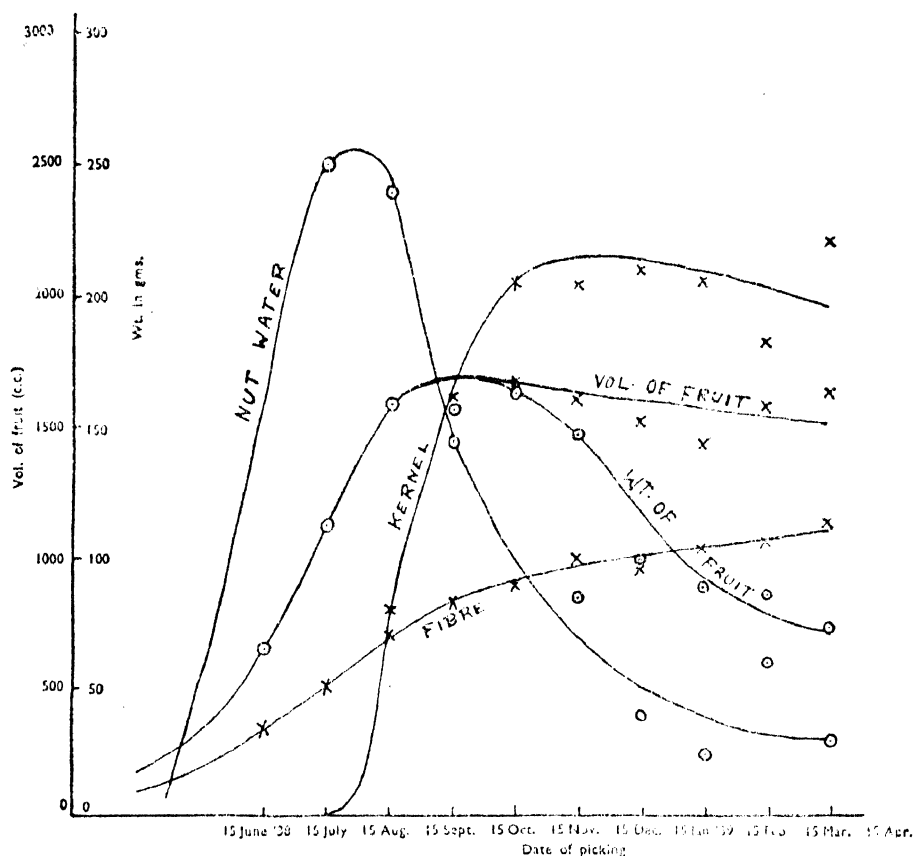


FIG. 2. Growth of fruit (Tree No. 152, Bunch No. 1)

Passing on to the remaining constituents of the coconut, Figs. 1 and 2 reveal several interesting features. The kernel is the last constituent to begin formation; and from the interpolated curves it is found that its formation does not commence during the first three or four months. Once it commences formation, its growth is of a 'blitz' nature for the first two or three months, during which time it gathers most of its raw material. Afterwards growth in the form of weight slackens, the kernel thickens and increases in density, and the formation of fat becomes vigorous. Ultimately, as the moisture content of the kernel falls, its gross weight also registers a decline. An analysis of the kernel at various stages of growth, similar to the one conducted in the case of coir fibre, was not carried out, as the author is exclusively confined to work on coconut husk. But there is ample evidence to conclude that the fat content of the kernel goes on increasing to the last days of growth of the fruit,

and that from the view-point of the oil industry the nuts are most advantageously gathered as late as possible. There is thus a conflict between the interests of the oil and coir industries. A compromise is the result, as practised in Malabar and the Southern Province of Ceylon, where the nuts are picked before they are fully ripe.

#### SUMMARY

Certain changes undergone by the various components of the coconut fruit during its growth from infancy to maturity have been traced by means of experiments performed over a period of about a year on select bunches of three different trees.

#### ACKNOWLEDGEMENT

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# ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY\*

## IX. THE ACID CHARACTER OF HYDROGEN CLAY

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**T**HE present paper is the concluding part of this series and gives a connected account of the experimental work relating to the acid character of hydrogen clay done in this laboratory. Theoretical considerations underlying this work have been discussed in an earlier paper [Mukherjee, Mitra and Mukherjee, 1937] and details will be avoided. It is, however, felt that a brief historical outline of the development on the theoretical side will be useful at this stage as the authors have not come across a comprehensive publication dealing with this aspect. Modifications of the current theoretical treatments are called for in the light of recent researches in this and other laboratories and it is intended to publish elsewhere a paper on these aspects.

### THE PHYSICAL AND CHEMICAL CONSTITUTION OF HYDROGEN CLAYS

The expression 'soil acidity' has been used to indicate, somewhat contrary to usage, the inherent acid character of soils. The nature and amount of the clay fraction determine to a large extent many of the properties of the soil, including its acid character. The hydrogen clay obtained from a soil represents the acid material in the clay fraction. From the point of view of physical and chemical constitution this clay fraction is a rather complex system. It consists of several chemical components in the sense of the phase rule some of which are not yet well defined and of particles whose size distribution varies widely from one clay to another. Their dimensions range from  $2\mu$  down to the lower limits of the colloidal range and the assemblage manifests colloidal properties.

Following the classical researches of van Bemmelen [1912] the inorganic colloidal material of the soil has often been regarded as a mixed gel of the oxides of iron, aluminium and silicon (also of manganese and titanium in much

\*Most of the results given in this series of papers have been taken from the Annual Reports for the years 1935-40 on the working of a scheme of research into the 'Properties of Colloid Soil Constituents' financed by the Imperial Council of Agricultural Research, India.

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smaller quantities); and the applications of the principles of colloid science to the study of soils has led to a rapid progress of our scientific knowledge regarding them. For a long time, however, this 'mixed gel' hypothesis and an erroneous stress on some aspects of colloidal properties appear to have kept in the background the crystalline nature of the inorganic soil colloids now definitely established by X-ray [Hendricks and Fry, 1930; Kelley, Dore and Brown, 1931; Hofmann, Endell and Wilm, 1934; Nagelschmidt, 1939], optical [Marshall, 1930, 1935; Hendricks and Fry, 1930] and thermal [Kelley, Jenny and Brown, 1936] investigations. Moreover, synthetic mixtures of purified colloidal oxides of silicon, iron and aluminium appear to differ materially from the clays obtained from soils [Bradfield, 1923].

Modern researches make it increasingly clear that the basis of a unitary treatment of the properties of the soil and clay lies in the recognition that they are essentially disperse systems with a dominant electrochemical (polar) character. Their colloid constituents belong to the class of electrolytic colloids.\*

The clay fraction as also the coarser particles which together build up the inorganic material of the soil are formed by the weathering of rocks which are polar or electrolytic substances. And the chemical reactions involved in weathering are mainly of an electrochemical character in which hydrolysis plays an important part. The influences controlling this hydrolysis, such as the reaction of the medium ( $pH$ ), the salt content, time of contact, degree of leaching and others are largely conditioned by climatic factors, e.g. rainfall and temperature and their seasonal variations. The weathered product is also in essence polar in character and carries with it the impress of the physical and chemical reactions which the parent rock has undergone. It is therefore not surprising that the importance of rather detailed investigations by physical and chemical methods of the colloid constituents of soil in soil genesis and soil classification is being increasingly realised [Byers *et al.*, 1931, 1932, 1933, 1935, 1936; Edelman, 1939].

In soils and clays the absorption complex consisting of the secondary clay minerals, comminuted primary minerals, humus and the free oxides is considered to be responsible for most of the colloidal properties manifested by them. Base exchange, flocculation, deflocculation, soil structure, shrinkage and swelling are illustrations of such properties which are determined by the nature and amount of the 'complex' present in the soil. The hydrogen clay represents the acid form of the complex freed from exchangeable bases. It is this acidic part which mainly determines the colloid behaviour of soil. When freed from organic matter, it represents the acid form of the inorganic part of the complex. It would thus appear that the electrochemistry of the colloid constituents, the clay and humus fractions of the soil, is of direct interest in the elucidation of many aspects of soil behaviour. And investigations on the electrochemical properties of the colloidal acid, the hydrogen clay, occupy a central place in a systematic treatment of soil behaviour.

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\*The expression 'colloidal electrolytes' should in our opinion be used exclusively for those electrolytic colloid systems where there is a thermodynamic equilibrium between the polymers and monomers. The expression 'electrolytic colloids' should be used in a more general sense so as to include, in addition to the above, colloidal systems where there is no such equilibrium but which show pronounced electrolytic properties.

The distinguishing characteristic of an acid is its power to combine with bases or to donate a proton to an acceptor. Colloidal acids and their salts have the additional characteristic of base exchange—a property which is not confined to soils, clays and other silicate minerals (e.g. bentonites, zeolites) or artificially prepared silicates (e.g. permutite) but is also exhibited by such organic substances as the acidic complexes of gums, resins and proteins.

Apart from base exchange these substances which differ so widely in their chemical composition and constitution show a number of points of resemblance. The acidic part as has been previously indicated is complex and has to be regarded as an anion of macro-dimensions. It carries a negative charge and shows the general characteristics of typical negatively charged colloids of which they constitute a special class distinguished by their stability and the large number of easily displaceable or reactive electrolytic ions associated per gramme of the material. The connecting link in the study of their common characteristics is consequently the fact that they are electrolytic colloids of an acidic nature with an electrical double layer surrounding their particles. The existence of such a double layer was postulated by Quincke and a picture of the electrical conditions inside the double layer was given by Helmholtz. Gouy [1910] formulated the existence of an atmosphere of diffuse ions which was in a sense implied in Helmholtz's mathematical treatment. The manner in which the electrical double layer is built up and the nature and distribution of the carriers of the electric charges which determine to a very large extent the behaviour of these systems have been set forth in detail by one of us [Mukherjee, 1921 ; 1922]. The recognition of the part played by the electrical double layer and by the ions constituting it has, however, come about gradually (further discussed later on).

#### THEORIES OF BASE EXCHANGE

The history of the development of our knowledge of hydrogen clays is mostly associated with the study of base exchange. It will be shown later that the interaction between a hydrogen clay and an electrolyte mainly consists in an exchange of H ions associated with the hydrogen clay for the cations of the electrolyte. Base exchange studies have thus an important bearing on studies of hydrogen clays. Earlier work on base exchange was, as the name of the topic suggests, concerned with the exchange of cations other than hydrogen ions. Exchange of the latter has come to be recognized more recently and systematic work is mainly associated with studies of hydrogen clays.

Way [1850] appears to have considered base exchange as an action involving double decomposition. Since Way, several attempts have been made to formulate this reaction on the basis of the law of mass action. Gans [1905] found that a permutite having the composition  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot n\text{H}_2\text{O}$  rapidly gave up its alkali metal in exchange for an equivalent quantity of another on being washed with its salt. The exchange was considered by him to be governed by the mass action equation  $K = \frac{x^2}{(m \cdot n - x)(g - x)}$  where  $K$  is the equilibrium constant ;  $m$ , the amount in grammes of the exchange complex ;  $n$ , the total amount in mols of exchangeable cations in the complex ;  $g$ , the total amount of the displacing ion in solution and  $x$ , the amount of it taken up by

base exchange. Rothmund and Kornfeld [1918] suggested the more general mass law equation  $\frac{c_1}{c_2} = K \frac{C_1}{C_2}$  where  $c_1$  and  $c_2$  are the concentrations of the two ions in the solid phase and  $C_1$  and  $C_2$  are the corresponding concentrations in the liquid, it being assumed that the added cation forms an isomorphous mixture with the solid so that there is only one solid phase. The generalized equation is in harmony with the observation often made [Wiegner and Muller, 1929] that the equilibrium is independent of the volume of the solution so long as the total amount of the displacing cation contained in it is constant. Equations based on the mass action principle have also been set up among others by Anderegg and Lutz [1923], Kerr [1928] and Vanselow [1932] using various simplifying assumptions. Marshall and Gupta [1933] have critically examined these equations and have shown that the equilibrium constant calculated from them has different values.

Further complications are met with when adsorption on the surface and reactions in colloidal systems have to be considered. Linder and Picton [1895] and Whitney and Ober [1901] observed that in the coagulation of colloidal solutions of arsenious sulphide by barium chloride, barium ions are carried down by the coagula. It has been shown more recently by Rabinovich [1925] and Weiser and Gray [1932] that hydrogen ions surrounding the colloidal particles are exchanged. Neither this exchange, nor its significance, was noticed for a long time. Van Bemmelen [1912] extensively studied adsorption of electrolytes by precipitates, e.g. of those of importance in chemical analysis. Lottermoser and Rothe [1908] in their well-known studies on silver salts also noted such adsorption. Marc [1911; 1913] came to the conclusion that adsorption of an ion by an insoluble polar solid, although weak, is of general occurrence when the ion can form an isomorphous and insoluble substance with one of the constituent ions of the solid [Fajans and Beckerath, 1921; Mukherjee and Basu, 1927]. An exchange adsorption or displacement adsorption gradually came to be recognized. Wiegner [1912] found that the exchange could in most cases be represented by Freundlich's equation  $\frac{x}{m} = KC^{1/p}$ , where  $m$  is the amount of the adsorbent in gramme;  $x$ , the amount adsorbed;  $C$ , the equilibrium concentration, and  $K$  and  $p$  are constants. Jenny's [1932] modified equation  $\frac{x}{m} = K \left( \frac{c}{a-c} \right)^{1/p}$  takes account of the fact that the exchange is often independent of dilution;  $a$ , in this equation, represents the initial concentration of the added salt. None of these parabolic equations, however, can explain the observation that at high concentrations of the added salt the exchange reaches a maximum. To meet this difficulty, Vageler [1931] proposed the hyperbolic equation  $Y = \frac{XS}{S+C}$  where  $Y$  is the amount taken up per gramme of the substance;  $X$ , the number of equivalents of the cation added per gramme of the adsorbent;  $S$ , the maximum exchange capacity and  $C$ , the number of equivalents of the added cation for which 50 per cent of  $S$  is exchanged. The Vageler equation agrees better at high concentrations with experimental results than the Wiegner equation. All these equations, however, are rather empirical and the various constants involved in them have little physical significance.

Jenny [1936] has recently suggested a simple base exchange model with the aid of which he has derived an equation based on statistical considerations.

He has considered a plane surface having a definite number of attraction spots per unit area. If the ions, atoms or molecules initially adsorbed on these spots are designated as  $b$  and the exchanging bodies as  $w$  then at equilibrium the number of  $w$  bodies adsorbed or released is given by the equation  $+(S+N) \pm \sqrt{(S+N)^2 - 4 S \cdot N \cdot (1 - V_w/V_b)}$  where  $N$  is the amount of electrolyte (number of ions) initially added;  $S$ , the saturation capacity; and  $V_w$  and  $V_b$ , the volumes of the oscillating spaces of the  $w$  and  $b$  bodies. The latter volumes are characteristic of the exchanging and exchanged bodies; and exchange adsorption takes place whenever the  $w$ 's slip in between the surface and the space occupied by the  $b$ 's which execute to and fro motions between the surface and a mean position in the bulk of the liquid. An almost perfect agreement between theory and experiment was observed by Jenny in certain cases while in others, systematic deviations occurred. Jenny sought to explain these latter on the basis of the structural peculiarities of the colloidal particles (discussed below) and the nature of the exchanging ions. Difficulties were encountered when hydrogen ions were involved and when the two ions participating in the exchange had widely different properties.

Further complications have been observed by later workers. For instance, Renold [1936] found a marked difference in the base exchange property of a permutite having the same composition and proportion of two exchangeable cations but prepared in different ways. Thus a mixed or 'hetero-ionic' permutite of given composition, e.g. one saturated with a fixed proportion of K and Ba ions can be obtained starting from either of two homo-ionic permutites saturated only with K or Ba ions. Renold [1936] observed that if the mixed permutite was obtained from the K-permutite its exchangeable K was more difficult to displace by a third cation than if it was prepared from a Ba-permutite.

The recognition of the part played by the electrical double layer in base exchange reactions, especially of soils and clays, originates from the work of Mukherjee [1922], Hissink [1924-25] and notably Wiegner [1925]. According to Wiegner, colloidal clay is made up of micelles consisting of an inner kernel which is an ultramicon, an inner layer of anions fixed on the surface and an outer swarm of cations. These cations can be displaced by others and the ease of displacement depends on the valency and radius (together with the hydration envelope) of the displacing cation and on the nature and the intensity of the force of attraction between the inner layer of anions and the cations already existing in the double layer. The replacing powers of different cations are given by their so-called 'symmetry values' which represent the 'ionic exchange in percentage when the number of ions added to the system is made equal to the total number of exchangeable ions in the colloidal complex'. Wiegner's fundamental ideas have since been extended by Jenny [1932], Marshall and Gupta [1933], Pallmann [1938] and others. The picture, however, is not yet complete in all its details. According to the views on the origin of the double layer formulated by one of us [Mukherjee, 1922] the base exchange clays have a layer of electrolytic ions, mainly anions,\* built up on the surface of their particle by lattice forces which constitute a primary

\*If the clay is amphoteric the primarily adsorbed layer will consist of both cations and anions.

layer of adsorbed ions ; an equivalent amount of oppositely charged ions (here, cations) remains associated with each particle partly as a fixed secondary layer and partly as ' mobile ' osmotically active ions\*. In the interaction with an electrolyte, the cations, fixed and mobile, already present in the double layer are liable to be exchanged for those of the electrolyte. The displacing power of different cations would depend on their valency and mobility, if their adsorption was the result of simple electrostatic forces alone [Mukherjee, 1922.]

#### SOME ASPECTS OF THE ACID CHARACTER OF SOIL

A quantitative formulation of the acid character of soil which, as stated before, is the main connecting theme in the electrochemistry of soil has long been lacking. The nature of the interaction between soil and neutral salts by which acid is liberated has been a subject of controversies. The main weakness of the purely chemical explanation according to which the reaction is a simple double decomposition process [Way, 1850 ; Truog, 1916 ; Page, 1926] lies in the assumption that the soil acid has to be considered to be unusually strong as, otherwise, it would not decompose a neutral salt combining with the base and liberating the strongest known acids, e.g. hydrochloric acid. Such an acid is evidently unknown and it is difficult to conceive of such reactions. On the other hand, the principal drawback of the purely physical explanation [Hopkins, 1903 ; Cameron, 1910 ; Parker, 1913] is that it links up the free energy of the process with an ill-defined surface energy term.

The theory of the double layer, on the other hand provides a more rational and simple explanation. According to the picture suggested by one of us [Mukherjee, 1922], ' an extract with a neutral salt can be acid when the cations displaced from the second sheet of the double layer contain hydrogen ions. ' Hissink [1924] also considered that hydrogen ions occur together with  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions in the outside sheet of the double layer. These  $\text{H}^+$  ions are also exchangeable. When we treat a soil with a solution of  $\text{KCl}$  the filtrate contains  $\text{H}^+$  ions. The following reaction takes place :—



Though the rôle of the double layer is now fairly generally realized, the above simple picture cannot explain several features. Thus, neutral salt extracts of acid soils almost always contain aluminium ions [Daikuhara, 1914]. Opinion differs as to whether  $\text{Al}$  ions are present in the outer sheets of the double layers and are directly exchanged for the cations of the salt, or, whether they are liberated by a process of secondary dissolution of a part of the alumino-silicate base exchange complex or the free alumina contained in it by the acid set free consequent on the exchange of the  $\text{H}$  ions of the double layer for the cations of the salt. Another observation which is not easy to understand is that an acid soil does not show a neutral reaction even on continued leaching with a solution of a neutral salt [Hissink, 1924-25]. Indeed, such difficulties led Hissink [1935] to remark that no existing theory

\*A distinction between fixed and mobile ions in the outer sheet of the double layer has not been made by Wigner. This distinction is justified by results of investigations on hydrogen clays discussed later.

can adequately explain the nature of the interaction between an acid soil and a neutral salt.

The nature of the acid-base interaction in soil cannot also be said to have been fully understood. There is much confusion regarding the part that the cation of the base plays in this interaction. Titration curves with different bases can rarely be superimposed and different amounts of these bases are required to attain a certain *pH* [Hissink and van der Spek, 1925 ; Oakley, 1927]. *Ad hoc* assumptions regarding the differences in the solubility of the 'salts' which the soil acid forms with different bases have been made [Truog 1916 ; Joseph, 1924 ; Oakley, 1927] to explain this non-equivalence, it being even necessary to postulate a wholesale existence of insoluble salts of alkali metals. Another observation for which an adequate explanation has not been forthcoming is that more base is necessary to attain a certain *pH* when the soil is titrated in the presence of a neutral salt than when titrated with the base alone [Crowther and Martin, 1925 ; Hardy and Lewis, 1929 ; Clark and Collins, 1930]. The soil buffer action is in fact an extremely complicated expression of several types of reactions in which anions (molecularly dispersed anions, e.g.  $\text{HCO}_3^-$  and  $\text{HPO}_4^{--}$  and macro-anions such as those of humus and the so-called zeolitic complex ions) and cations (e.g.  $\text{Al}^{+++}$  and  $\text{Fe}^{+++}$  ions) of a number of weak acids and bases take part. A buffer action although comparatively much weaker may be shown by the free inorganic oxides contained in the soil. Further, the possibility of a total absorption of the base as suggested by Oakley [1927] and Mattson [1931] cannot be altogether ignored. A quantitative formulation of the soil buffer action comprehending at least the chief factors has not been forthcoming. The gaps in our basic knowledge of the subject have been responsible for the element of arbitrariness in the various routine methods in vogue for estimating the so-called lime requirement and the base exchange or base-binding capacity of soil. These methods seldom give concordant results (further discussed later). Unlike the estimation of acids in true solution, or colloidal systems in which the different phases and components taking part in the interaction can be clearly defined [Mukherjee, 1922 ; 1929], the amounts of acids estimated by these methods are usually ill-defined and there is often no clear idea as to what is being estimated by a particular method.

#### THE SCOPE AND OBJECT OF THIS WORK

Considerable light has been thrown in recent years on problems of soil acidity by investigations (discussed more fully later) on hydrogen clays and systems resembling them carried out, among others, by Wiegner and associates at Zurich, Anderson, Brown and Baver in America, Mattson at Upsala, Marshall in England, Hissink and coworkers and Edelman and associates in Holland. Definite and adequate information, however, is still lacking on several fundamental points (mentioned below). Our work aims to secure this information. Specially oriented technical procedures, described in the previous parts of this series (see, in particular, part IV [Mukherjee *et al.*, 1936] ; part V [Mitra 1936] ; and part VII [Mitra, 1940], have been used which have yielded more accurate results than are usually aimed at in soil investigations. Also, the problems have been approached from a point of view which differs from previous investigations of a similar nature. Instead of studying

only the properties of hydrogen clays, simpler but similar systems which are amenable to straightforward theoretical treatment have been examined. They were expected, on theoretical grounds, to be more suitable for the study of the processes underlying the interaction of hydrogen clay with electrolytes. The main objective of the work on hydrogen clay has been the elucidation of the following topics :

- (1) The electrochemical character of hydrogen clay ;
- (2) the manner in which this electrochemical character varies : (a) with the nature of the soil from which the hydrogen clay has been isolated, (b) with the particle size of the hydrogen clay, and (c) on the removal of the free silica and sesquioxides contained in the hydrogen clay ;
- (3) the base exchange capacity of hydrogen clay and the factors on which it depends ; and
- (4) the weathering process.

The work on hydrogen clays is being now extended to sub-fractions of hydrogen clays, clay minerals of standard purity and the so-called clay salts\* and these latter investigations will be dealt with in separate series of papers.

Modern electrochemistry gives in terms of the concepts of activity coefficient and ionic strength a satisfactory representation of the interaction between an acid and a base barring complications arising out of other types of chemical reactions which have of course to be taken into account. In order, therefore, to understand the nature of hydrogen clay, it was considered necessary, firstly to ascertain how far its behaviour can be brought within the compass of our current concepts of electrochemistry ; secondly, to ascertain features, if any, which show that they have properties which cannot be comprehended by these concepts ; and thirdly, to formulate a picture which would enable us to understand these special characteristics and reconcile them with the usual concepts.

The properties of so complex and variable a system as the hydrogen clay can be properly understood only by systematic studies of hydrogen clays obtained from a sufficiently large number of different types of soils. Those used for this work were obtained from different parts of the country (details given in Table I) and had widely different mechanical and chemical compositions and base exchange properties. The results summarized in this paper show that in spite of these variations, the hydrogen clays and sub-fractions of hydrogen clay obtained from them reveal a number of important common features though individual differences are not lacking. The present paper is mainly concerned with the common features. Individual differences have also been indicated ; they have been more fully discussed in part VIII [Mukherjee, Mitra *et al.*, 1942].

#### EXPERIMENTAL

Details regarding the method of preparation of the hydrogen clays, experimental arrangements and procedure have been given in parts IV, V and VII. The following soils and bentonites\*\* were used.

\* i. e. clays with exchangeable cations other than  $H^+$  ions

\*\*Bentonites usually have chemical composition and base exchange properties similar to those of soil. The samples of bentonite used for this work were kindly supplied by the Assam Oil Company

TABLE I

*Particulars of soils, bentonites, hydrogen clays and hydrogen bentonites used*

Lab. No.	Description of soil or bentonite	Silica : sesquioxide ratio (molar) of entire clay fraction	Ref. No. of corres- ponding hydrogen clay or hydrogen bentonite
13	Brownish yellow soil (unmanured) from Gov- ernment Farm, Suri (Bengal) collected at a depth of 6-12 in. from Agricultural Chemist's experimental plot, block A 1-16, plot Nos. 3, 5, 16	2.34	E
14	High land acid soil from Government Farm, Burdwan (Bengal), collected at a depth of 0-6 in. from block B, plot No. 40 of the Farm	1.94	F
20	Neutral calcareous soil (brown loam) from Government Seed Farm, Kalyanpore (U.P.), collected at a depth of 0-6 in.	2.10	H
25	Black cotton soil (neutral, calcareous) from Satara (Bombay), collected at a depth of 0-6 in.	2.50	I
32	Neutral black soil from Bilaspur, near Raipur (C. P.), collected at a depth of 0-6 in.	2.54	K
22	Red laterite soil (acidic) from Government Farm at Dacca (Bengal) collected at a depth of 0-6 in.	1.99	L
34	Black soil from Government Farm, Akola (Berar), collected from a depth of 0-9 in.	2.19	M
33	Bhata red laterite soil from Raipur (C. P.), collected at a depth of 0-6 in.	1.88	N
46	Non-lateritic black calcareous soil (B-type) from Government Farm at Padegaon (Nira, Poona), collected at a depth of 0-12 in.	2.51	Padegaon-B
51	Acid soil from Government Farm at Jorhat (Assam), collected at a depth of 0-6 in.	2.58	Jorhat-F
53	High land acid soil on old alluvium from Government Farm at Latekujan (Assam), collected at a depth of 0-6 in.	2.47	Latekujan-F
B. O. C.1	Bentonite from Hati-Ki-Dhani . . . .	2.86	Hati-Ki-Dhani-B
B. O. C.3	Bentonite from Bhadres . . . .	2.90	Bhadres-B

## RESULTS

*Colloidal solutions of hydrogen clay as heterogeneous acid systems.*

The interpretation of the electrochemical properties, especially the titration curves, of an acid system would largely depend on whether the system is a single, or polyphase system. Colloidal solutions of hydrogen clays have often been regarded as homogeneous acid systems. Bradfield [1923 ; 1927] and Bayer [1930] have attributed to them a weak monobasic acid character from a study of their titration curves. The so-called 'suspension effect' of Wiegner and Pallmann [1929] according to which a suspension of an insoluble acidic substance, e.g. a hydrogen clay, has a much higher H ion activity than the pure dispersion medium indicates, on the other hand, a polyphase character of such an acid system. Doubts have recently been expressed regarding the validity of the suspension effect [Rabinowitsch and Kargin, 1935]. Experiments carried out during the past few years in this laboratory with carefully purified colloidal solutions of hydrogen clays fully bear out this effect.

Some results are given in Table II.

TABLE II

*Free and total acids of hydrogen clay sols and their ultrafiltrates*

Sol	pH	Free acid H-ion conc. $\times$ $10^5 N$	Total acid* $\times 10^5 N$
E . . . . .	4.66	2.19	24.3
Ultrafiltrate . . . . .	6.10	0.08	Nil
F . . . . .	4.41	3.89	38.0
Ultrafiltrate . . . . .	5.90	0.13	Nil
G . . . . .	4.57	2.69	40.0
Ultrafiltrate . . . . .	5.85	0.14	**
H . . . . .	4.52	3.02	99.0
Ultrafiltrate . . . . .	6.05	0.09	**

\*The total acidities have been calculated from the inflexion points in the potentiometric titration curves of the sols with baryta. It will be shown later that this value does not denote the concentration of the total neutralizable acid as the added base continues to react beyond the inflexion point.

\*\*Not determined

The ultrafiltrates of the sols are practically free from any acid, while the sols have a fairly low pH. The existence of mobile hydrogen ions giving rise to pH's of the order of 4.5 is, therefore, beyond doubt.

*The special features of some simple polyphase acid systems : Difficulties of their interpretation in the light of the classical concepts*

Reference has already been made to the extremely complex nature of hydrogen clay as a chemical entity and as an acid system. For a proper understanding of its properties investigations on simple polyphase systems, e.g. colloidal silica, alumina, and palmitic and stearic acids have been carried out and the results discussed in parts I, II, III and IV [Mukherjee *et al.*, 1931 ; 1932 ;

1934; 1936] and in other publications [Mukherjee, Mitra and Mukherjee, 1937; Mukherjee, 1937; Dutta 1939; Chatterjee 1939]. These investigations have served a twofold purpose. They have brought to light a number of characteristic features of heterogeneous acid systems which are foreign to the concepts of classical electrochemistry; secondly, they have served as a guide to the investigations on hydrogen clay. A connected account of a part of the work on the simple systems has been given by Mukherjee, Mitra and Mukherjee [1937]. The following is a summary of the more important observations which have a direct bearing on the work on hydrogen clay discussed in this paper.

A simple heterogeneous acid system where the part played by the solid phase can be readily understood is illustrated by the mixture of a solid acid and its saturated solution. Fig. 1 reproduced from part I gives the titration curves of saturated solutions of cinnamic acid in presence (curves 2 and 3) and absence (curve 1) of the solid acid.

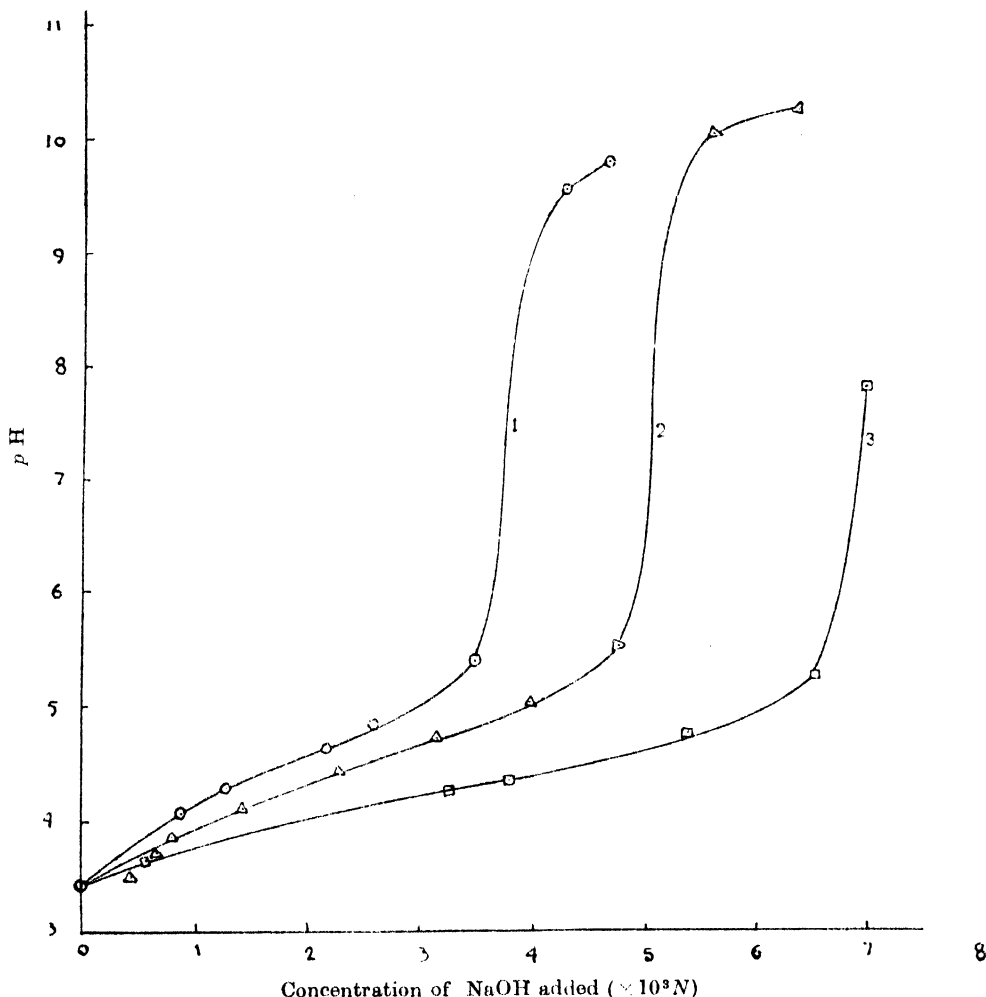


FIG. 1. Titration curves of saturated solutions of cinnamic acid in the presence and absence of excess solid acid

The amount of the acid reacting with the base (NaOH) increases so long as solid cinnamic acid is present. The resulting salt, sodium cinnamate, remains in solution. The equilibrium  $pH$  on the addition of a definite amount of the base can be calculated from the volume of the aqueous phase and the solubility and dissociation constant of cinnamic acid. The actual  $pH$  during the course of the titration, i.e. the form of the titration curve, is, however, determined not merely by these equilibrium\*values but by the kinetics of the interaction between the solid acid and the base. The net effect of the presence of the solid phase is a prolongation of the initial portion of the titration curve (Fig. 1, curves 2 and 3) where the  $pH$  does not change so rapidly as it does in the case of the saturated solution containing no solid acid (Fig. 1, curve 1). The initial portion of the curve thus resembles that of a stronger acid. As opposed to a strong acid in true solution, however, the influence of increasing concentrations of the salt in suppressing the dissociation of the acid manifests itself in the region of higher  $pH$  values. When the solid phase disappears the titration curve resembles, subject to the influence of the higher anion concentration, that of the saturated solution. If, however, one attempts to calculate the dissociation constant from different points of curves 2 and 3 using the well-known Henderson equation, constant values are not obtained. This arises from the fact that the fundamental assumption underlying the derivation of the equation that the whole amount of the reacting acid exists in true solution is not satisfied when the solid phase is present. The variability of the total acid under this latter condition leads to values of the dissociation constant thus calculated which are fictitious and denote quantities which have not the usual significance.

If a colloidal solution of a hydrogen clay were an acid system which gave two insoluble solid phases —a solid acid and a solid salt, its behaviour as deduced from classical considerations would be represented by the titration curves of palmitic and stearic acid sols [Mukherjee, 1937; Iyer, 1932; Datta, 1939]. The titration curve of a stearic acid sol with baryta is reproduced from Datta's paper (Fig. 2).

The curve shows an initial rise, then a middle horizontal portion at a practically constant  $pH$  between 6.2 and 6.3 followed by a sharp inflexion. These features can be explained by the phase rule. Barium stearate is insoluble in water. On the addition of barium hydroxide the  $pH$  rises till the solubility product of barium stearate is reached. So long as the insoluble salt separates out as a second solid phase, the system which has three components (barium hydroxide, stearic acid and water) and exists in four phases (the solid acid, the solid salt, the liquid and the vapour) is univariant. At constant temperature, therefore, the liquid phase should have a constant composition. The horizontal portion of the curve confirms this expectation. When the solid acid phase has disappeared the system becomes bivariant and consequently the  $pH$  shoots up on further addition of the base. During the titration with baryta, the barium stearate molecules continually split off from the surface and form a crystal lattice of their own. Inner layers are, therefore, continually exposed to the action of the alkali and ultimately the whole of the acid takes part in the reaction. The total acid at the inflexion point has been found to agree with the amount of acid in the sol estimated on extraction with ether.

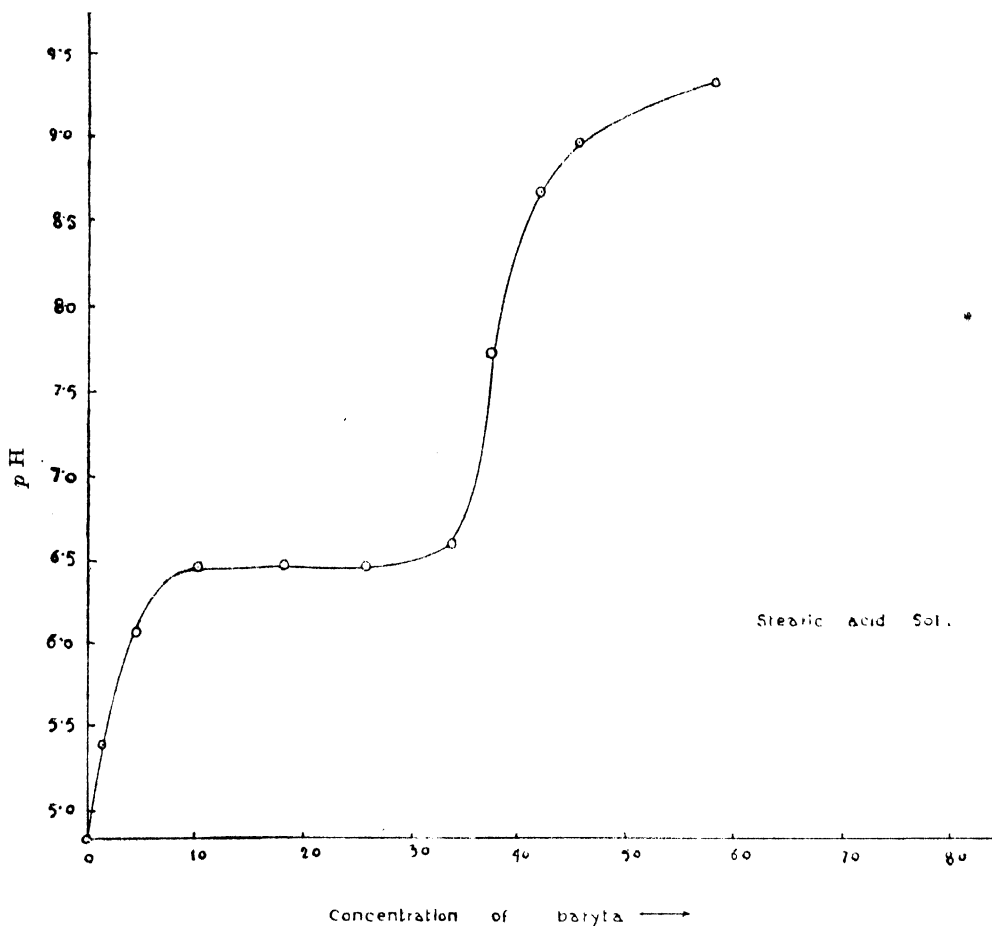


FIG. 2. Titration curve of a stearic acid sol with baryta

The above simple heterogeneous acids have the advantage that the nature of the surface reactions can be visualized and the amount of the acid solid phase entering into the reaction can be calculated when equilibrium has been established. In most colloidal solutions of acidic substances, however, we have no *a priori* knowledge of the quantities necessary for the calculation, namely the solubility, the anion concentration and the dissociation constants. In the case of an acid in the dissolved condition, the cations and anions into which it dissociates are both present in a state of true solution. But with a colloidal solution of an acid, e.g. silicic acid sols, the position is very much different. Silicic acid when freshly formed appears to be present in a state of true solution but rapidly polymerizes, giving rise to colloidal silicic acid. In parts II, III and IV (also Chatterjee [1939]) it has been shown that the sols give rise to hydrogen ion activities of the order of  $10^{-4}N$ . The free and total acids of the ultrafiltrates constitute a small fraction (about 10 per cent or even less) of those of the corresponding sols. Colloidal solutions of silicic acid, therefore, possess an intrinsic acid character, which is independent of the presence of foreign substances. The potentiometric titration curves of the sols with bases show

inflexion points in the acid region, between  $pH$ 's 4.3 and 5.4. Some typical titration curves are given in Fig. 3 reproduced from Chatterjee's [1939] paper.

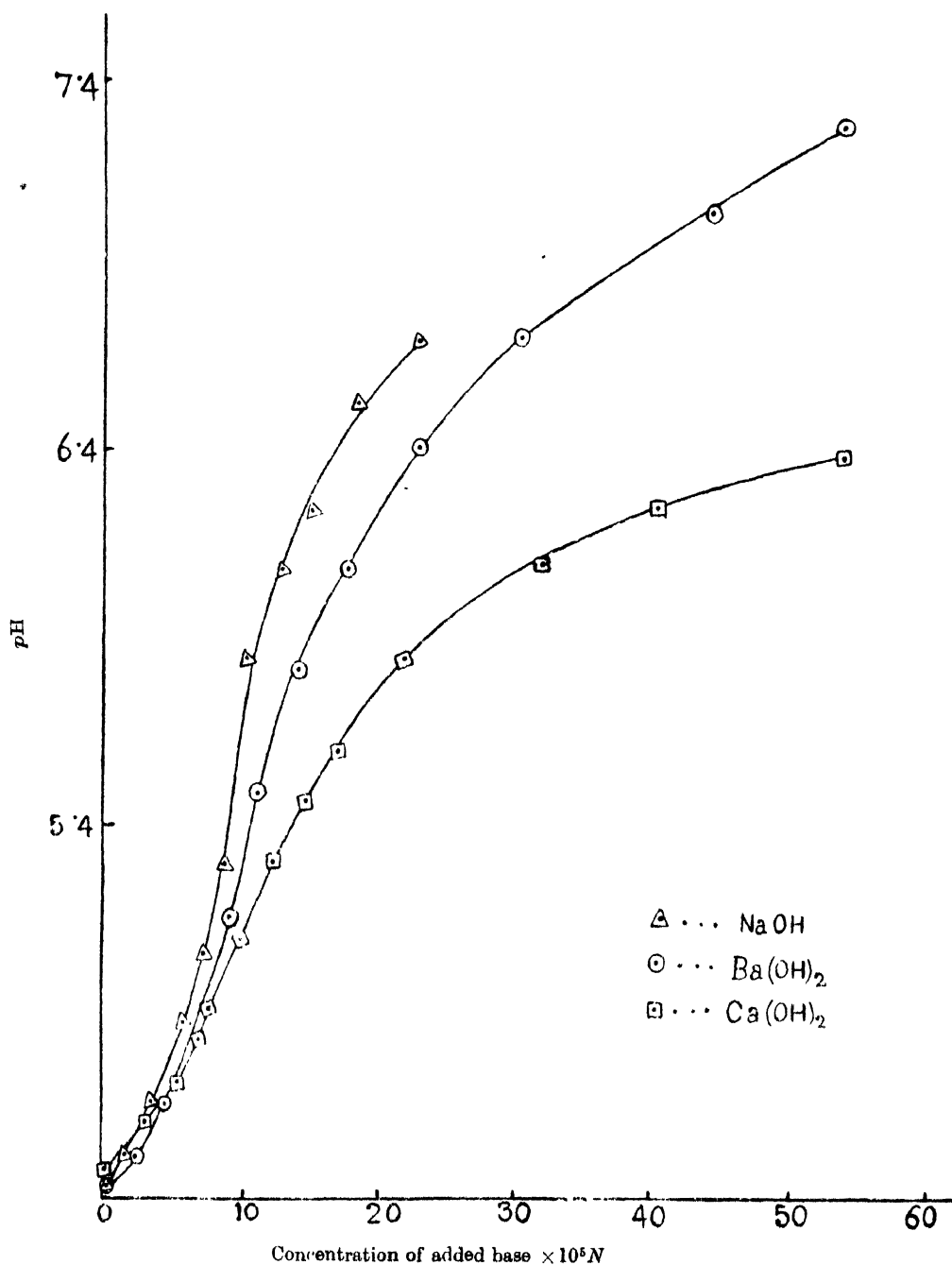


FIG. 3. Titration curves of silicic acid sol

The location of the inflexion point in the acid region is very significant and in true solutions of acids would imply that the titrated solution contains either a mixture of several acids of different dissociation constants and/or a polybasic acid. The negligible free and total acids of the ultrafiltrates indicate the absence of any dissolved acid. Further, though not so significant, the titration curves do not show a second inflexion point till a  $pH$  of about 11.0 is reached. The inflexion point in the acid region is definitely characteristic of the colloidal solution of the acid. It signifies that there is a constant amount of hydrogen ions surrounding the colloidal particle at a definite level of affinity. A portion of these is free and is responsible for the observed e.m.f. of a hydrogen electrode and the remaining portion is 'bound'.

Different dilute bases give almost the same total acid when calculated from the first inflexion point in the corresponding curves. The amount of hydrogen ions neutralized at the inflexion point is thus a fixed quantity. The slopes of the titration curves show that the intensities with which different bases react with the sol are in the order\*  $Ca(OH)_2 > Ba(OH)_2 > NaOH$ . The greater capacity of  $Ca(OH)_2$  compared with  $Ba(OH)_2$  to react with the sol confirms the greater insolubility of calcium silicate compared with barium silicate observed by Joseph and Oakley [1925]. The degree of dissociation, if considered to be given by the ratio of the free acidity to the total acidity at the first inflexion point, has values between 0.5 and 1.0.

The amounts of acid liberated by salts (added as chlorides) as shown by the diminution in the  $pH$  are in the order  $Ba > Ca > Na$ .\*\* On repeated leaching of silicic acid sols with a given concentration of a salt the amount of acid in the leachate gradually diminishes. The total amounts of acid obtained in the leachates are greater for  $BaCl_2$  than for  $CaCl_2$  at the same concentration of both. Moreover, both salts liberate much more acid than is obtained at the first inflexion point in the titration with the bases alone. It thus appears that 'bound' hydrogen ions are present in addition to those which react with the base at the first inflexion point. These hydrogen ions are, therefore, held at the surface at a higher energy level. But the total quantity of the corresponding so-called salt that is formed by continued leaching is a small fraction of the number of moles of  $SiO_2$  present. The reaction with the salt does not lead to the formation of a second solid phase and is limited to the surface. The maximum amount of acid which thus reacts with  $N-BaCl_2$  has been found to constitute about 0.13 per cent of the total silicon dioxide.

The greater relative effect of Ba than Ca in the interaction with the salts and in the acid region is definitely against the explanation that the development of acidity is due to the formation of insoluble silicates. It has already been mentioned that calcium silicate is more insoluble than barium silicate and consequently the order should be  $Ca > Ba$ , i.e. the reverse order of what has been observed. *Ad hoc* assumptions regarding changes of the relative order of solubilities of the barium and calcium salts in the acid region consequent on the formation of another type of salt or the existence of a different type of acid are therefore necessary from the usual chemical point of view to

\* This order represents what has been called the irregular cation effect.

\*\* This order represents what has been called the regular cation effect as it agrees with the lyotrope series

explain these observations. The cation effects observed with silicic acid sols become more pronounced with hydrogen clays and soils. (Further discussed later).

Titration curves of silicic acid sols have been found to show a second inflexion point at a higher  $pH$  value between  $pH$ 's 11.0 and 11.8, depending on the concentration of the sol. The total acid calculated from this inflexion point shows a fair agreement with the silica content (gm. mols. per litre) of the sol. The composition of the resulting salt at the inflexion point is  $Na_2O \cdot 2SiO_2$ . The buffer-capacity curves show only one maximum, near about the point of half neutralization and it is justifiable to conclude that the salt formed is  $NaHSiO_3$ . But the maximum value of the buffer capacity is considerably greater than that observed in the case of a dissolved acid having the same dissociation constant and total acidity. The greater buffer capacity arises from a continuous solution of the colloidal particles which act as a reservoir from which fresh quantities of the acid are supplied. Colloidal silica behaves in its interaction at a high  $pH$  as a dibasic acid, its first dissociation constant is about  $5.2 \times 10^{-10}$  and its solubility of the order of 0.045 gm. of  $SiO_2$  per litre at  $30^\circ C$ . Colloidal silicic acid thus constitutes a heterogeneous phase, of which a part, the charged colloidal particle, is definitely not in a state of true solution. The interaction is limited to the interface unless the  $pH$  is high enough to dissolve the particles and to hold the resulting silicate ions in true solution. The reaction is then no longer restricted to the interface and conforms to the usual type of chemical reaction between an insoluble acid and a base.

#### *The electrochemical properties of hydrogen clay sols*

Far greater complexities have been observed with hydrogen clays. They are discussed below. It will be shown that the theory of the double layer as postulated by one of us [Mukherjee, 1921; 1922] provides a satisfactory explanation of their special features. The following simplified picture\* of the double layer may be postulated for hydrogen clays. There is a primarily adsorbed layer of anions, presumably  $OH$  and  $O$  ions as indicated by X-ray [Kelley and Jenny, 1936] and other [Bar, 1935] investigations, built into the surface. An equivalent amount of hydrogen ions and, as will be shown later, aluminium ions, is held near the surface. A part of these  $H$  ions may be fixed by electrostatic forces or bound on the surface by chemical valence or Van der Waals type of forces. The remainder are osmotically active. They constitute the mobile sheet of the double layer and carry an electric charge equal and opposite to that of the free anions on the surface. The 'bound' ions are present in an osmotically inactive condition. The mobile hydrogen ions give rise to the observed  $H$ -ion activity of the sol. On the addition of an electrolyte, an interchange takes place in the first instance between the cations of the electrolyte and the mobile  $H$  ions of the double layer. These cations can also displace the bound  $H$  ions and are themselves adsorbed by simple electrostatic forces and/or by specific forces (chemical valence, or Van der Waals forces). What has been distinguished as electrical adsorption of ions carrying a

\* It takes no account of the amphoteric character of hydrogen clay stressed by some workers [Mattson, 1932; 1937].

charge of the opposite sign to that of the primarily adsorbed ions is determined by electrostatic forces as given by their valency and diameter in the state of hydration in which they occur on the surface, each of them forming an 'ion pair' with a primarily adsorbed ion on the surface. The state of hydration (or of dehydration) of the ion is of importance in relation to the energy of the ion pair. In electrical adsorption the ion pair contains the oppositely charged ion (here, the cation) in the same state of hydration as in the solution. The lyotrope series of cations,  $\text{Th} > \text{Al} > \text{Ba} > \text{Sr} > \text{Ca} > \text{Mg} > \text{Rb} > \text{K} > \text{Na} > \text{Li}$ , follows from these considerations. When adsorption is brought about by chemical or other specific forces, the energy of the resulting ion pair depends on its chemical properties and the latter consequently determine the intensity of adsorption. Probably in the latter case the cation is adsorbed in a dehydrated condition. The various types of exchange observed by us between the H ions of the double layer and cations of added electrolytes and their effects on the free and total acids of the sols and the form of their titration curves are discussed below.

*Interchanges between H ions and cations of added salts*

If only the mobile H ions are exchanged for the cations of the salt, no marked alteration in the H-ion activity of the sol will take place. That of the ultrafiltrate of the sol, on the other hand, will show a considerable increase proving that H ions have been displaced from the double layer into the intermicellary liquid. The following results illustrate this point.

TABLE III

*pH of hydrogen clay sols and their ultrafiltrates in the presence and absence of salts of alkali metal cations*

System	pH
Sol H . . . . .	4.52
Ultrafiltrate of sol H . . . . .	6.05
Sol H + 0.0005N KCl . . . . .	4.26
Ultrafiltrate of above . . . . .	4.42
Sol H + 0.002N KCl . . . . .	4.10
Ultrafiltrate of above . . . . .	4.15
Sol Padegaon-B . . . . .	4.54
Ultrafiltrate of sol Padegaon-B . . . . .	5.85
Sol Padegaon-B + 0.0005N NaCl . . . . .	3.85
Ultrafiltrate of above . . . . .	4.35

In the above experiments, the weak electrical adsorption of the K and Na ions and the low concentration of the salts are responsible for the

exchange being restricted to the mobile H ions alone. The variation in the H-ion activity of the sols is consequently negligible.\* Much larger variations indicating a displacement of the bound H ions are observed on adding larger concentrations of salts, specially salts of alkaline earth metals as the following results will show.

TABLE IV  
*Decrease of pH of hydrogen clay sols on the addition of different salts*

Sol	Original pH	Salt added	Conc. of salt	Decrease of pH
E . . . . .	4.66	NaCl	0.10N	1.05
		CaCl <sub>2</sub>	..	1.14
		BaCl <sub>2</sub>	..	1.51
H . . . . .	4.52	NaCl	0.80N	1.06
		CaCl <sub>2</sub>	..	1.19
		BaCl <sub>2</sub>	..	1.29
I . . . . .	4.51	CaCl <sub>2</sub>	0.25N	1.08
		BaCl <sub>2</sub>	..	1.20

The marked lowering of the pH of the sol on the addition of the salts indicates a displacement of mobile as well as bound H ions. Under similar experimental conditions practically no change in pH is observed with a solution of HCl having nearly the same pH as the sols. The relative effects of the different cations to liberate acid follow the order Ba > Ca > Na which is in agreement with the lyotrope series and the order of electrical adsorption of cations. In the interaction of hydrogen clay with neutral salts, therefore, the cation effect is determined by electrostatic forces alone. It is a regular cation effect.

Similar to what has been observed with silicic acid sols, the reaction between the hydrogen clay and the neutral salts does not proceed to completion. The quantity of the so-called 'clay salt' formed as given by the amount of the cation fixed by the hydrogen clay is less than the total quantity of acid associated with the hydrogen clay as obtained on titrating it with a base, especially an alkaline earth hydroxide, in the presence of a salt.

The sol and salt mixture contains : (i) free H ions displaced into the intermicellary liquid from the double layer, and (ii) H ions, free and bound, associated with the flocs contained in the mixture†. When the mixture is titrated, the free H ions are first neutralized and then as the pH rises more and more H ions are displaced from the flocs in presence of the salt to maintain equilibrium and neutralized. The large number of cations present in the system materially helps this process and the cation effect is emphatically shown up by the fact that compared to the sol itself, the sol and salt mixture has a much larger total acid or base exchange capacity (b. e. c.) measured at the inflexion

\* The slight lowering of the pH of the sol arises from a displacement of some bound H ions from the double layer

† The part played by aluminium ions in determining the free and total acids of hydrogen clay and salt mixtures will be discussed later

point of the titration curve or at a fixed pH, e.g. 7.0. The higher the concentration of the salt in the mixture, the greater is the b. e. c. In the presence of a fixed concentration of different salts the b. e. c. decreases in the order  $Ba > Ca > Na$  in agreement with the regular cation effect. The results given in Tables V and VI illustrate these points. The cation effect is really responsible for the observation often made that more base is required to attain a certain pH when the titration is carried out in the presence of a salt than in its absence.

The H ions which are brought into a neutralizable condition on the addition of the salt to the hydrogen clay sol are not all displaced into the intermicellary liquid. This is shown by the results given in Tables VI and VII. A smaller b. e. c. is obtained on titrating the clear supernatant liquid above the coagulum of the sol and salt mixture and the extract obtained on repeatedly leaching the sol with the solution of the salt than on titrating the mixture itself.

TABLE V

*Base exchange capacity in m. c. base per 100 gm. of oven-dried (105°C.) hydrogen clay using NaOH, Ba (OH)<sub>2</sub> and Ca(OH)<sub>2</sub>*

System	NaOH		Ba(OH) <sub>2</sub>		Ca(OH) <sub>2</sub>	
	At inflexion point	At pH 7.0	At inflexion point	At pH 7.0	At inflexion point	At pH 7.0
Sol E . . . . .	2.2(5.4)*	15.4	20.6(6.0)	25.0	21.5(5.8)	26.2
Sol E + 0.1N NaCl	16.1(5.0)	26.4	..	..	..	..
.. + 0.1N BaCl <sub>2</sub> .	..	..	28.0(4.6)	>42.2	..	..
.. + 0.1N CaCl <sub>2</sub> .	..	..	..	..	21.2(4.4)	40.6
Sol L . . . . .	16.3(8.21)	6.30	17.5(7.10)	17.0	19.0(6.6)	19.5
Sol L + 1.0N NaCl .	22.3(7.0)	22.3	..	..	..	..
.. + 1.0N BaCl <sub>2</sub> .	..	..	32.0 (5.4)	40.5	..	..
.. + 1.0N CaCl <sub>2</sub> .	..	..	..	..	30.0(6.1)	32.5
Sol N . . . . .	18.8(7.5)	11.3	19.0(7.0)	19.0	20.5(6.4)	21.8
.. + 1.0N NaCl .	21.25(6.0)	22.5	..	..	..	..
.. + 1.0N BaCl <sub>2</sub> .	..	..	28.5 (5.5)	35.0	..	..
.. + 1.0N CaCl <sub>2</sub> .	..	..	..	..	23.0(5.4)	26.5

\* The figures within brackets denote the pH at the inflexion point

TABLE VI

*Base-exchange capacities calculated from the titration curves of the sol, the sol + salt mixture and the clear supernatant liquid above the mixture, and the amounts of the cation of the salt adsorbed by the hydrogen clay before neutralization with the base*

Conc. of BaCl <sub>2</sub> added	B. e. c. at pH 7.0 in m. e. Ba(OH) <sub>2</sub> per 100 gm. colloid obtained on titrating			M.e. Ba adsorbed per 100 gm. colloid
	Sol H	Sol H + BaCl <sub>2</sub> ( <i>in situ</i> )	Supernatant liquid of sol H + BaCl <sub>2</sub>	
0	32.0	..	..	..
0.01N	..	33.0	11.4	11.1
0.02N	..	35.0	11.9	12.7
0.04N	..	37.5	14.4	15.2
0.09N	..	43.5	17.0	18.5
1.0N	..	48.0	22.0	24.2

TABLE VII

*Base-exchange capacities obtained on titrating the sol + salt mixture, and successive portions of the salt extract*

System titrated	B. e. c. at pH 7.0 in m. e. Ba (OH) <sub>2</sub> per 100 gm. colloid
sol H + 0.83N BaCl <sub>2</sub>	48.0
1st 100 c.c. of leachate	27.0
2nd " " "	3.0
3rd " " "	1.2

The last column of Table VI shows the amount of the cation (Ba) adsorbed by the hydrogen clay from the solution. This amount is, as is to be expected, in fair agreement with the b. e. c. calculated from the titratable acid in the supernatant liquid of the sol and salt mixture given in the fourth column of Table VI.

The above results show that routine methods in which neutral salt extracts of acid soils are titrated for estimating their exchangeable hydrogen and lime requirement give only a fraction of their total neutralizable acid or b. e. c. owing to an incomplete displacement of the H ions of the double layer by the cations of the salt. The back reaction set up by the H ions which have been already displaced in the intermicellary liquid is responsible for this incomplete

exchange. When, however, the sol and salt mixture is titrated *in situ*, these latter H ions are continuously removed from the sphere of action and the neutralization is further helped by the large number of cations present in the system.

*Interchanges between H ions and cations of added bases*

(1) *Variations of the b. e. c. obtained on titration with different bases.\*—* Cation effects also play a definite rôle when the sols alone are titrated with bases. In titrating the sol, apart from the direct neutralization of the free H ions by the OH ions of the base, its cations displace various amounts of bound H ions from the double layer which are then neutralized by the OH ions. The greater the displacement, the greater is the amount of acid reacting with the base at a fixed pH. Also, the higher the pH, the greater is the total acid or the base-exchange capacity (b. e. c.) with a given base. Titration with different bases gives different b. e. c.'s calculated both at the inflexion point in the titration curves as also at pH 7.0 as the results given in Tables V and VIII will show.

TABLE VIII

*Base-exchange capacity of hydrogen clay calculated from titration curves with different bases*

Sol	Base used for titration	pH at inflexion	B. e. c. in m. e. base per 100 gm. colloid	
			At inflexion point	At pH 7.0
H	NaOH	5.4	2.2	15.4
	Ba(OH) <sub>2</sub>	6.0	20.6	25.0
	Ca(OH) <sub>2</sub>	5.8	21.5	26.2
	Ba(OH)	5.8	21.5	32.0
	Ca(OH) <sub>2</sub>	6.6	21.5	32.8
	NaOH	8.05	90.0	78.0
	Ba(OH) <sub>2</sub>	7.00	82.0	82.0
K	Ca(OH) <sub>2</sub>	6.95	96.0	97.0
	NaOH	7.15	68.0	61.0
	Ba(OH) <sub>2</sub>	5.80	55.0	67.0
	Ca(OH) <sub>2</sub>	5.20	58.0	67.0

\* Some results illustrating the variability of the b. e. c. of hydrogen clay have been discussed in part VI [Mitra, Mukherjee and Bagchi, 1940]

The b. e. c. of E, L and N calculated at the inflexion point as also at pH 7.0 decreases in the order  $\text{Ca(OH)}_2 > \text{Ba(OH)}_2 > \text{NaOH}$ . This is the order in which the b. e. c. at pH 7.0 of I and K varies. That of I calculated at the inflexion point follows the order;  $\text{Ca(OH)}_2 > \text{NaOH} > \text{Ba(OH)}_2$ . With K, the order is  $\text{NaOH} > \text{Ca(OH)}_2 > \text{Ba(OH)}_2$ . In comparing the b. e. c. with different bases the pH at which it is measured is an important factor. The inflexion points do not all occur at the same pH. The titration curves from which the b. e. c.'s have been obtained show that increasing amounts of the base react with the sol as the pH rises. The comparison should, therefore, be made at the same pH, e.g. pH 7.0. At this pH, the b. e. c. in all cases decreases in the order  $\text{Ca(OH)}_2 > \text{Ba(OH)}_2 > \text{NaOH}$ . The slopes of the titration curves also point to the same order of the capacity of the different bases to react with the hydrogen clays.

(2) *Features of titration curves.*—The cation effects also markedly influence the form of the titration curves. The study of these titration curves constitutes one of the important steps in the elucidation of the electrochemical character and the results previously obtained by us have been described in parts V, VI, VII and VIII. Bradfield [1927] was the first to obtain the titration curves of electrodialysed hydrogen clays and following him, the work has been continued by Bayer [1930], Bayer and Scarseth [1931], Denison [1933] and others. Bradfield observed that the curves 'were of the type to be expected of very weak acids' and showed an inflexion point at pH 8.5 for NaOH and pH 7.0 for  $\text{Ba(OH)}_2$ . He found that both the bases gave the same b. e. c. at the respective inflexion points. Bayer [1930] found a constant b. e. c. with all strong bases. But it will be seen from his paper that the pH at inflexion decreases in the order  $\text{LiOH} > \text{NaOH} > \text{KOH} > \text{Ba(OH)}_2 > \text{Ca(OH)}_2 > \text{Mg(OH)}_2$ . According to Bradfield and Bayer this was the order in which the corresponding salts of the clay acid were hydrolysed in an aqueous medium, but one might ask for the reason of the difference in the behaviour of these strong bases. The conductometric titration curves of Bradfield and Bayer also resembled that of a weak monobasic acid. Bradfield, on the whole, considered that the reaction between a hydrogen clay and a strong base is an ordinary neutralization process and that 'recourse to the adsorption theory seems unnecessary'. However, some of his own observations are certainly difficult to explain in the light of the simple picture suggested by him. Thus, while the end point of his conductometric titration curve with caustic soda gave the same total acid as the inflexion point of the potentiometric titration curves with this base and baryta, the end point of the conductometric curve with baryta gave a much larger total acid and this curve had also a somewhat different shape compared with the conductometric curve with caustic soda. Its initial portion was flatter and the end point more rounded. To explain these differences, Bradfield postulated the formation of insoluble products by the interaction of hydrogen clay with alkaline earth hydroxides. It is, however, difficult to see how the formation of such insoluble products would affect the forms of the potentiometric curves in the manner described by him. A simple parallel case is illustrated by the formation of calcium citrate from citric acid and calcium hydroxide. On the gradual addition of the base to the acid more and more calcium citrate will be precipitated and the pH will very slowly rise till all the acid has been neutralized. Further addition of the base will result in a sharp rise of the pH as

would be observed if the base were added to water. The titration curves of stearic acid sols with baryta or lime (Fig. 2) illustrate the case where the weak acid and the resulting salt are both almost insoluble. The features characteristic of the above two cases are not shown by the titration curves of hydrogen clay obtained by previous workers and by us (discussed below). For example, the inflexion point is not as sharp as might be expected and the curves indicate a pronounced buffer-action beyond the inflexion point. According to Bradfield [1927] 'this latter feature is difficult to explain'. The following observations made by us further illustrate the difficulties in the way of accepting a true weak monobasic acid character of hydrogen clay sols as postulated by Bradfield and others.

In contrast to the sharp initial rise of the potentiometric titration curve with NaOH (Fig. 4) which indicates a weak acid character, the conductometric titration curve with this base shows a comparatively sharp minimum, characteristic of the titration of a strong or a moderately strong acid by a strong base. Such a minimum would not be observed if the sodium salt of the clay acid underwent a marked hydrolysis. On the other hand, the conductivity should increase from the beginning of the titration as actually observed by both Bradfield and Bayer. In our work with about 40 hydrogen clays and sub-fractions of hydrogen clay having silica/alumina ratios ranging from 1.87 to 4.43 and prepared from soils of widely different origin, a sharp minimum has been observed without exception in the conductometric titration curve with NaOH. It is possible that Bradfield and Bayer missed this minimum owing to too large quantities of the alkali being added from the beginning of the titration. We have observed that the minimum in the conductometric titration curve with NaOH gives only a small fraction of the base-exchange capacity, usually 10-20 per cent, calculated from the inflexion point of the corresponding potentiometric titration curve, which lies between pH 7.0 and 8.5\*. Beyond the minimum the conductometric titration curve shows a break and the b. e. c. calculated from this break agrees with that obtained from the inflexion point of the potentiometric titration curve. The conductivity minimum is real and cannot be referred to the neutralization of any dissolved acid or acids present as impurity in the sol as the titration curve of the ultra-filtrate of the sol shows no such minimum. In fact, the ultrafiltrate contains little or no titratable acid (Table II). Its pH ranges from 5.8 to 6.8 according to the degree of purification of the sol, while that of the sol lies between 4.2 and 4.8 in agreement with the 'suspension effect' of Wiegner and Pallmann [1929]. The pH of the sol depends on the amount of the solid material contained in a given volume of the sol.

A second objection against the 'weak acid' theory is the fact that the dissociation constant  $K$  calculated from different points of the NaOH curve (which shows the greatest resemblance with that of a weak acid) with the aid of the well-known Henderson equation has been found to have different values [Mitra, 1940]. The concentration of the salt was taken as equal to that ( $B$ ) of the base added and the concentration of the unneutralized undissociated

\* The potentiometric titration curve with NaOH of some hydrogen clays, e.g. that of F shown in Fig. 5 shows an inflexion point in the acid region in addition to the one in the alkaline region (discussed later). In such cases, the minimum of the conductometric curve gives nearly the same total acid as the inflexion point in the acid region.

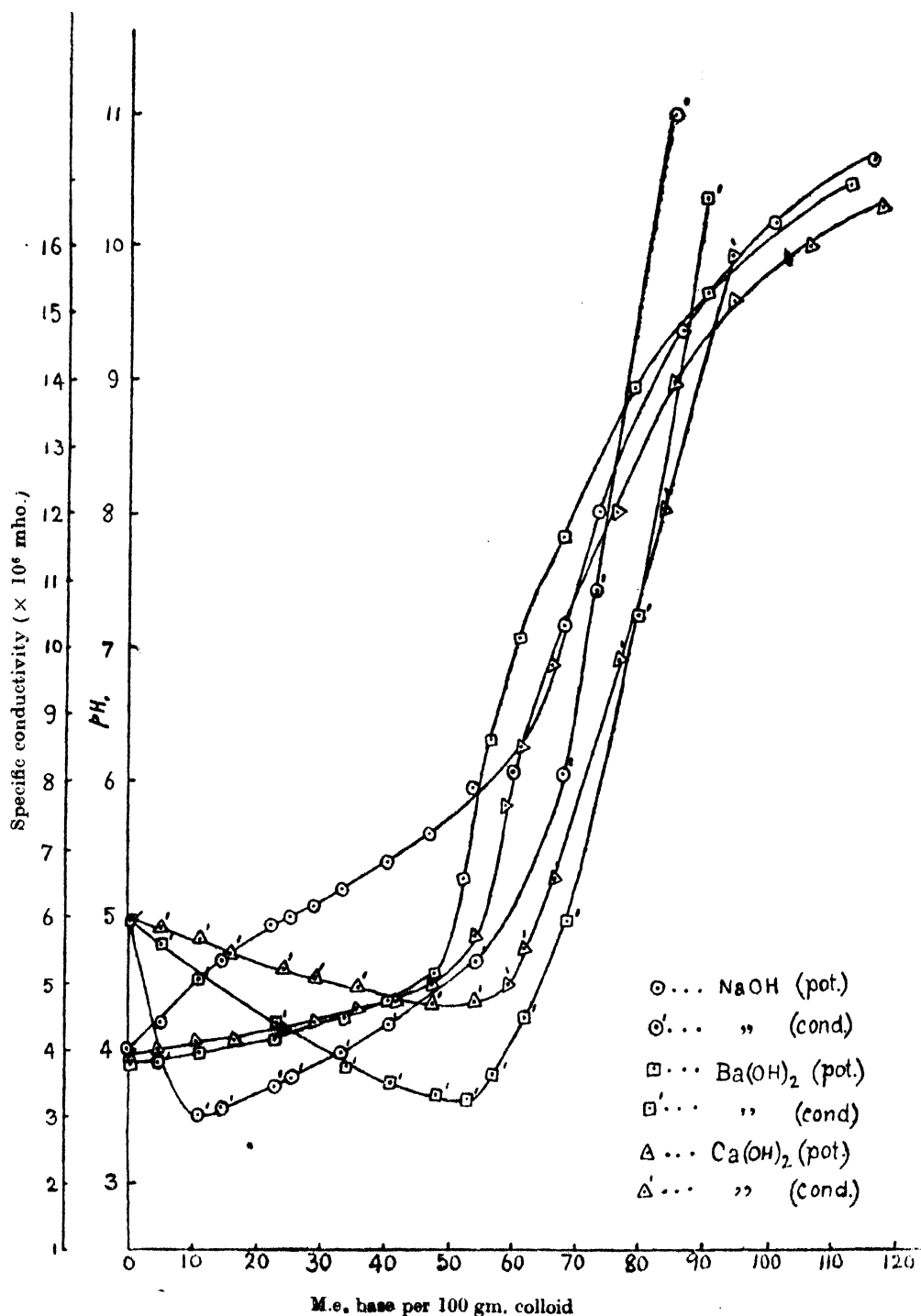


FIG. 4. Potentiometric and conductometric titration curves with NaOH, Ba(OH)<sub>2</sub> and Ca(OH)<sub>2</sub>

acid as equal to  $C-B$  where  $C$  is the total acid \* given by the inflexion point in the titration curve. Further, the dissociation constant calculated in the above manner does not agree with that given by the other mass action equation

$$K = \frac{\alpha^2 c}{1-\alpha}$$

where  $\alpha$  is the ratio of the free acid of the sol to the total acid  $C$

and may therefore be called the degree of dissociation of the sol.  $\alpha$  has a very small value even at total acid concentration of the order of  $10^{-4} N$  which is in agreement with the weak acid character of the NaOH curve (potentiometric). But a strong acid character of the sol is indicated by its potentiometric titration curves with  $\text{Ba(OH)}_2$  and  $\text{Ca(OH)}_2$ .

Some of our potentiometric titration curves with NaOH show a dibasic acid character. Those of hydrogen clays F and Latekujan-F and hydrogen bentonite Hati-Ki-Dhani-B show this feature. (Fig. 5)\*\*

The first inflexion occurs near about pH 5.0 and the second between 7.0 and 8.0. The initial portion of the titration curve of F has the appearance of that of a strong acid, and the weak acid character of the NaOH curve (potentiometric) of K shown in Fig. 4 is absent. These individual differences in the form of the titration curves are likely to be useful in the characterization and classification of soils [Anderson and Byers, 1936]. They have been more fully dealt with in part VIII [Mukherjee, *et al.*, 1942]. They further show that an error might easily be made in drawing any general conclusion regarding the acid character of hydrogen clay unless supported by observations on a sufficiently large number of hydrogen clays prepared from soils of widely different origin and type.

Of about 50 hydrogen clays and their sub-fractions studied by us the potentiometric titration curves with alkaline earth hydroxides of only one hydrogen clay, N, and two sub-fractions of L and the hydrogen bentonite Bhadres-B showed a weak acid character. The titration curves with these bases of all other hydrogen clays have a slowly rising initial portion which shows a more or less marked inflexion point as would be observed in the case of a strong or moderately strong acid. The corresponding conductometric titration curves, however, show a weak acid character (Figs. 4 and 6). These mutually conflicting features of the two sets of titration curves are not possible to reconcile if the interaction between the hydrogen clay and the base is considered to be a simple neutralization process.

The  $\text{Ba(OH)}_2$  and  $\text{Ca(OH)}_2$  curves of the majority of the hydrogen clays give an inflexion point between pH's 5.5 and 6.3; for a minority the inflexion point lies between pH's 6.3 and 7.0, and in a few cases the inflexion point has been found in the weakly alkaline region although the initial portion of the titration curve shows a strong acid character. The occurrence of the inflexion point near about pH 7.0 observed with some hydrogen clays is consistent with the strong monobasic acid character of the titration curves. Its occurrence in the acid region which is more common indicates, according to usual concepts of electrochemistry as pointed out in the case of silicic acid sols, that the hydrogen clay is either a mixture of several dissolved acids having different

\*This total acid given by the inflexion point does not represent all the neutralizable acid present in the system

\*\*Sub-fractions of Hati-ki-Dhani-B and Latekujan-F also have a dibasic acid character

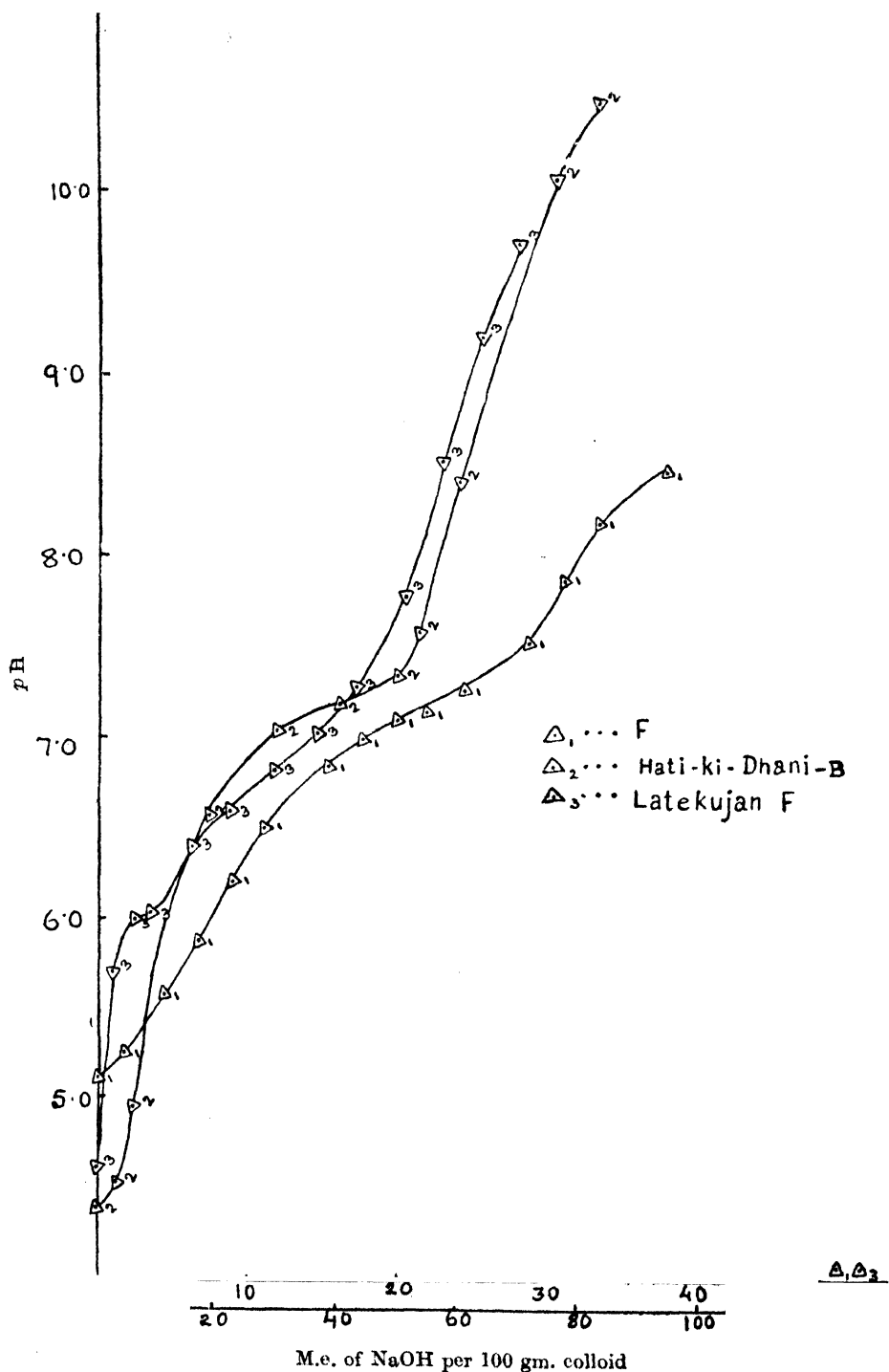


FIG. 5. Potentiometric titration curves with NaOH of 'dibasic' hydrogen clays and hydrogen bentonites

dissociation constants or it is a polybasic acid. The fact that the ultrafiltrate of the sol contains negligible free and total acids rules out the first possibility. The sol cannot also be considered to be an ordinary polybasic acid whose various dissociation constants have markedly different values as the titration curve shows no second inflexion even when the titration is extended to pH 12.5\*. Above this pH, the hydrogen clay is likely to decompose.

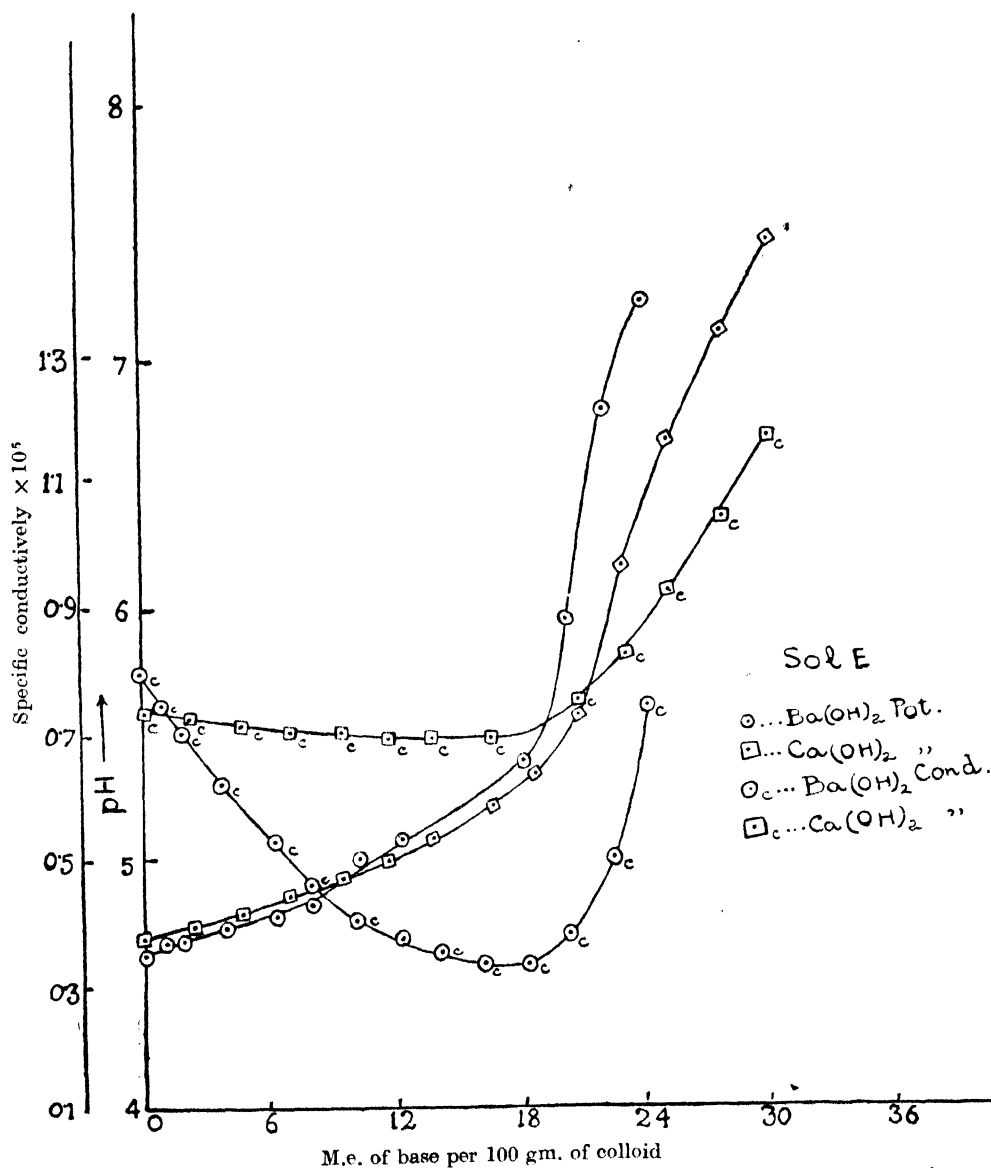


FIG. 6. Potentiometric and conductometric titration curves of hydrogen clay with Ba(OH)<sub>2</sub> and Ca(OH)<sub>2</sub>

\* Of course, if the dissociation constants do not differ much from one another, other inflexions will not be observed

The picture postulating the existence of mobile and bound H ions on the surface and their exchange for the cations of an added base or salt provides a consistent explanation of the slopes of the titration curves including the apparently contradictory features of the potentiometric and conductometric curves discussed above. The first additions of the base neutralize the mobile H ions. This displaces the equilibrium between mobile and bound H ions which is restored by the passage of some bound H ions from the bound to the mobile condition. Adsorption of the cations of the base facilitates this process. When barium or calcium hydroxide is the base used, the Ba or Ca ions, because of their strong electrical adsorption, displace more and more bound H ions from the beginning of the titration and the H ions thus displaced are neutralized by the OH ions of the base. The titration curve (potentiometric) has, therefore, a flat run (Figs. 4 and 6) indicating a moderately strong acid character. When the limit to which the bound H ions can be so displaced (by the cation of the base used) and neutralized has been reached, further addition of the base results in a sharp rise of the pH, that is the titration curve shows an inflexion point. This limit, however, does not correspond to the neutralization of all the bound H ions. And the titration curve shows a continued buffer action beyond the inflexion point. Also, as previously shown, titration in the presence of a large concentration of a neutral salt yields a much larger b. e. c. The inflexion point in the titration curves with bases thus indicates the neutralization of H ions up to a definite affinity level.

Using sodium hydroxide also, the first additions of the base would similarly neutralize the mobile H ions. The bound H ions which far outnumber the mobile H ions\* cannot be easily displaced from the double layer by the Na ions because of their weak electrical adsorption. The pH of the system, therefore, shoots up and the titration curve shows a comparatively sharp initial rise. On further addition of the base, the concentration of Na ions in the system increases and with it, their adsorption on the surface. This, combined with the gradually increasing pH of the system, helps in the neutralization of more and more bound H ions and the titration curve shows a flattening after the initial rise. When the limit to which the bound H ions can be so displaced and neutralized has been reached, further addition of the base results in a sharp rise of the pH, that is, the titration curve shows an inflexion. Fig. 4 (also Figs. 8 and 9) illustrates these features.

The electrical adsorption of the cations of the base also influences the slopes of the conductometric curves and the considerations set forth above can account for their features. The greater the adsorption the more bound H ions will be displaced and neutralized at a given pH and the slope of the curve will resemble that of a weak acid. The  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  curves (conductometric) given in Figs. 4, 6 and 7 show these features.

The displacement of bound H ions and their neutralization would also diminish the slope of the potentiometric titration curve but in this case a smaller slope indicates a stronger acid. The NaOH curve (conductometric) has the greatest downward slope though the corresponding potentiometric curve shows the steepest initial rise, thus indicating the weakest acid character.

\*This is shown by our observation that hydrogen clay sols have a small ratio (less than 10 per cent) of the free acid to the total acid calculated from the inflexion point in the titration curve with a dilute base

The slopes (of the descending portions) of the conductometric curves are arranged in the order:  $\text{NaOH} > \text{KOH} > \text{NH}_4\text{OH} > \text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2$  (Fig. 7). The slopes observed with sol F have been compared with the theoretical\* slopes in Table IX.

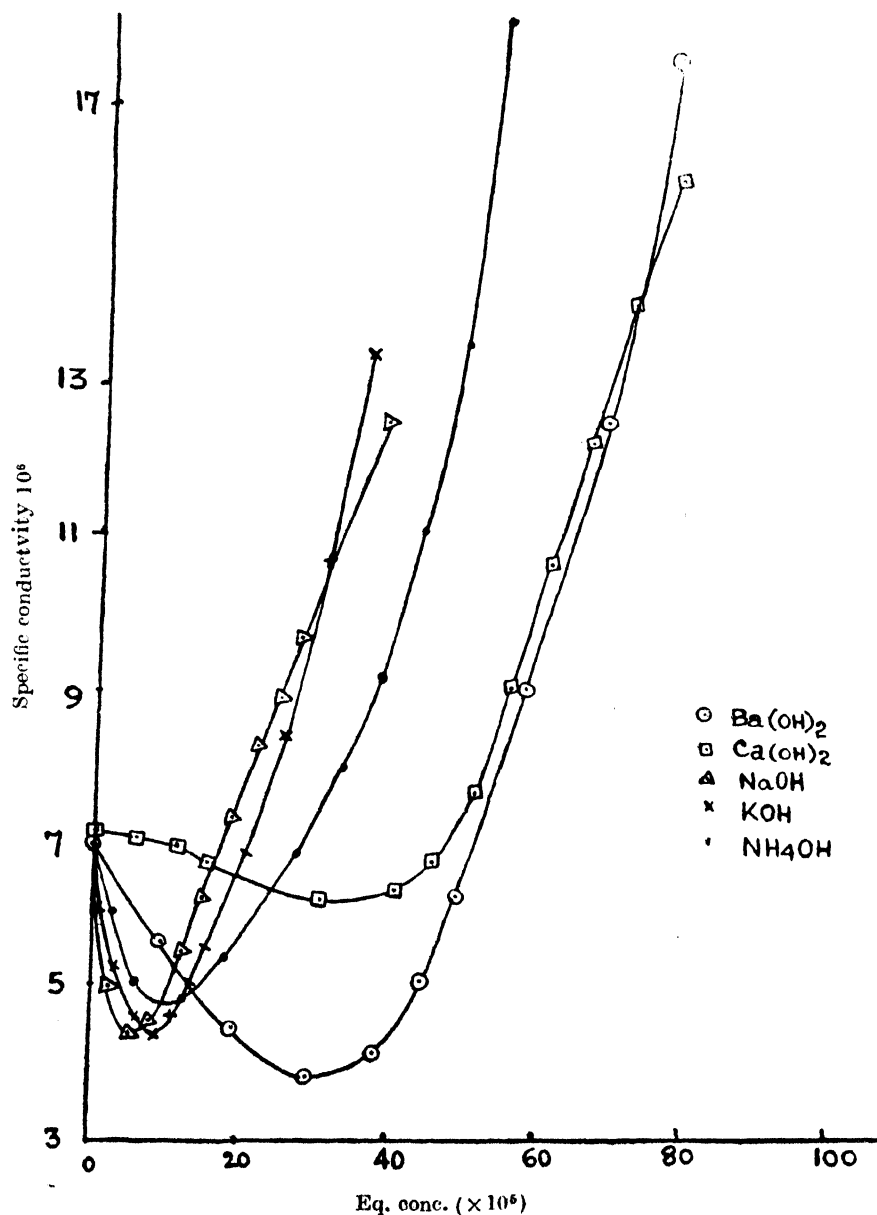


Fig. 7. Conductometric titration curves of hydrogen clay with  $\text{NaOH}$ ,  $\text{KOH}$ ,  $\text{NH}_4\text{OH}$ ,  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$

\* The theoretical slope is given by  $(U_{\text{H}^+} - U_{\text{M}^+})/1000$  where  $U_{\text{H}^+}$  and  $U_{\text{M}^+}$  are respectively the mobilities of  $\text{H}^+$  ion and  $\text{M}^+$  the cation of the base

TABLE IX

*Observed and calculated slopes of conductometric titration curves of hydrogen clay with various bases*

Base used	Slope of conductometric curve	
	Observed	Calculated
NaOH . . . . .	0·100	0·346
KOH . . . . .	0·085	0·318
NH <sub>4</sub> OH . . . . .	0·045	0·315
Ba(OH) <sub>2</sub> . . . . .	0·018	0·330
Ca(OH) <sub>2</sub> . . . . .	0·003	0·335

The observed slope is always less than the theoretical one. The greatest discrepancy is marked with the Ba(OH)<sub>2</sub> and Ca(OH)<sub>2</sub> curves, which arises from the strong electrical adsorption of the cations of these bases. The relative slopes of these two curves confirm the conclusion previously made that in the interactions with the bases alone Ca ions have a stronger effect than Ba ions.

#### *Regular and specific cation effects*

The main features of the cation effects described in the previous section and their importance in routine methods of estimation of the b. c. c. of soils and hydrogen clays are discussed below.

The effect of cations in determining the lime requirement and base-binding capacity of soil has been recognized by several workers [Crowther and Martin, 1925 ; Hardy and Lewis, 1929 ; Clark and Collins, 1930 ; Mattson, 1932 ; 1935]. The exact nature of the cation effect, however, has not been clearly defined. Our results show that this cation effect varies in certain respects with the pH and the distinction made in the previous section between the regular and specific cation effects takes account of this variation. The regular cation effect observed in the interactions with the salts and with bases in the presence of salts operates in the acid region. The sol and salt mixture contains a considerable amount of free acid, its pH being often as low as 3·2 depending on the nature and concentration of the salt added, while that of the sol itself lies near about pH 4·5. The interaction with bases in the presence of salts also mainly takes place in the acid region, usually between pH's 3·5 and 5·5, and consequently here also the regular cation effect is observed. The titration curve of the sol and salt mixture starts from a pH near about 3·5, has a flatter run than the titration curve of the sol with the same base and usually shows an inflexion point between pH's 4·5 and 5·5 (Table V and Figs. 8 and 9).

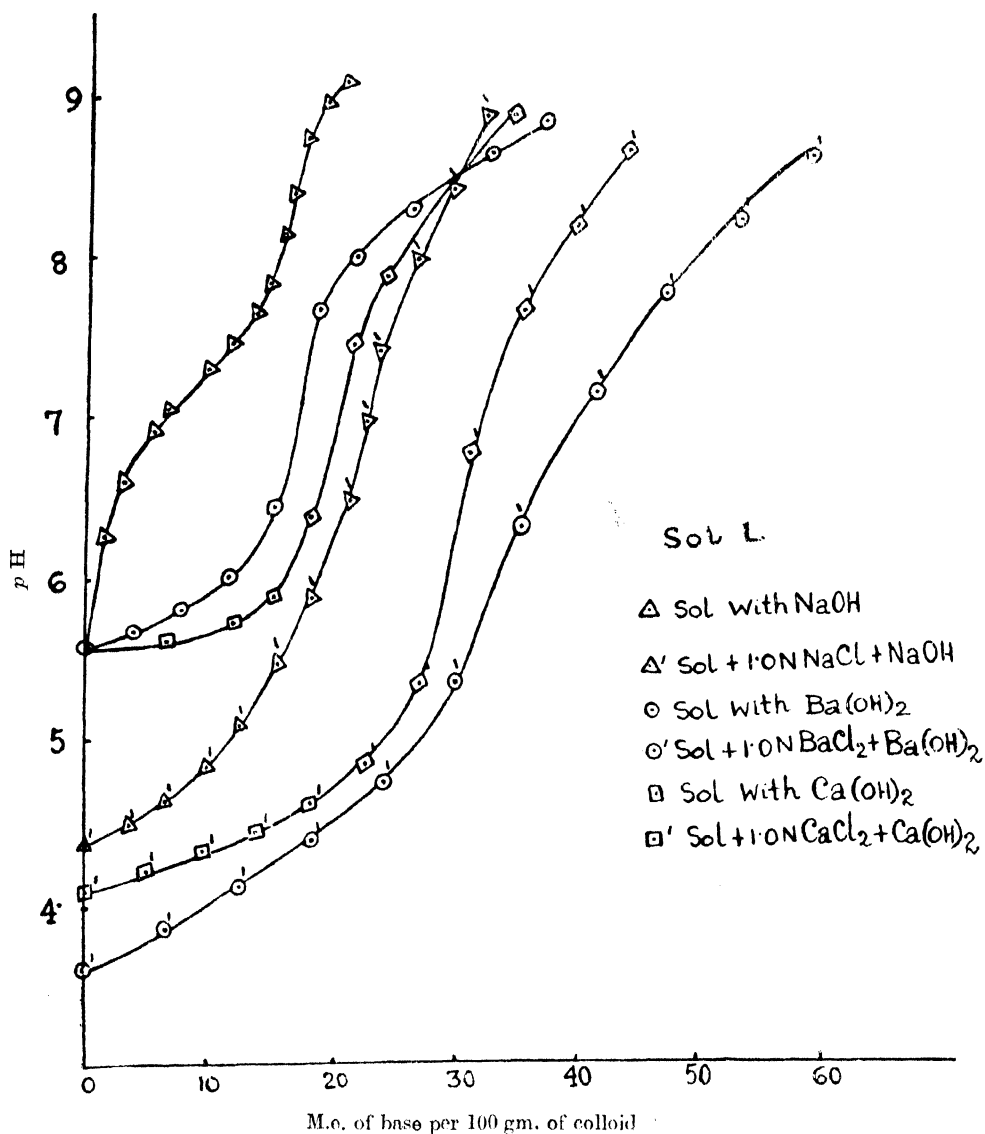


FIG. 8. Potentiometric titration curves of hydrogen clay L in the presence and absence of salts

The specific cation effect observed in the interactions with the bases, on the other hand, operates in the weakly acid to alkaline region. The titration curves with the bases, especially those with NaOH, are less buffered in the acid region compared with the titration curves of the sol and salt mixtures and their inflexion point occurs at a much higher pH, usually between 5.5 and 8.5 (Table V).

Reference has been previously made to the lack of agreement between the values of the base-exchange capacity (b. e. c.) of soil obtained by various routine methods. Using a number of hydrogen clays, comparison of different routine

methods shows [Mitra and Mitra, 1940; Mukherjee *et al.*, 1942] that this disagreement arises from the fact that sometimes the  $pH$  and, more generally the cation effects which operate in the several methods are not the same in a quantitative sense. Concordant results have, however, been obtained when the same type of cation effect is involved at the same  $pH$  and equilibrium conditions are considered.

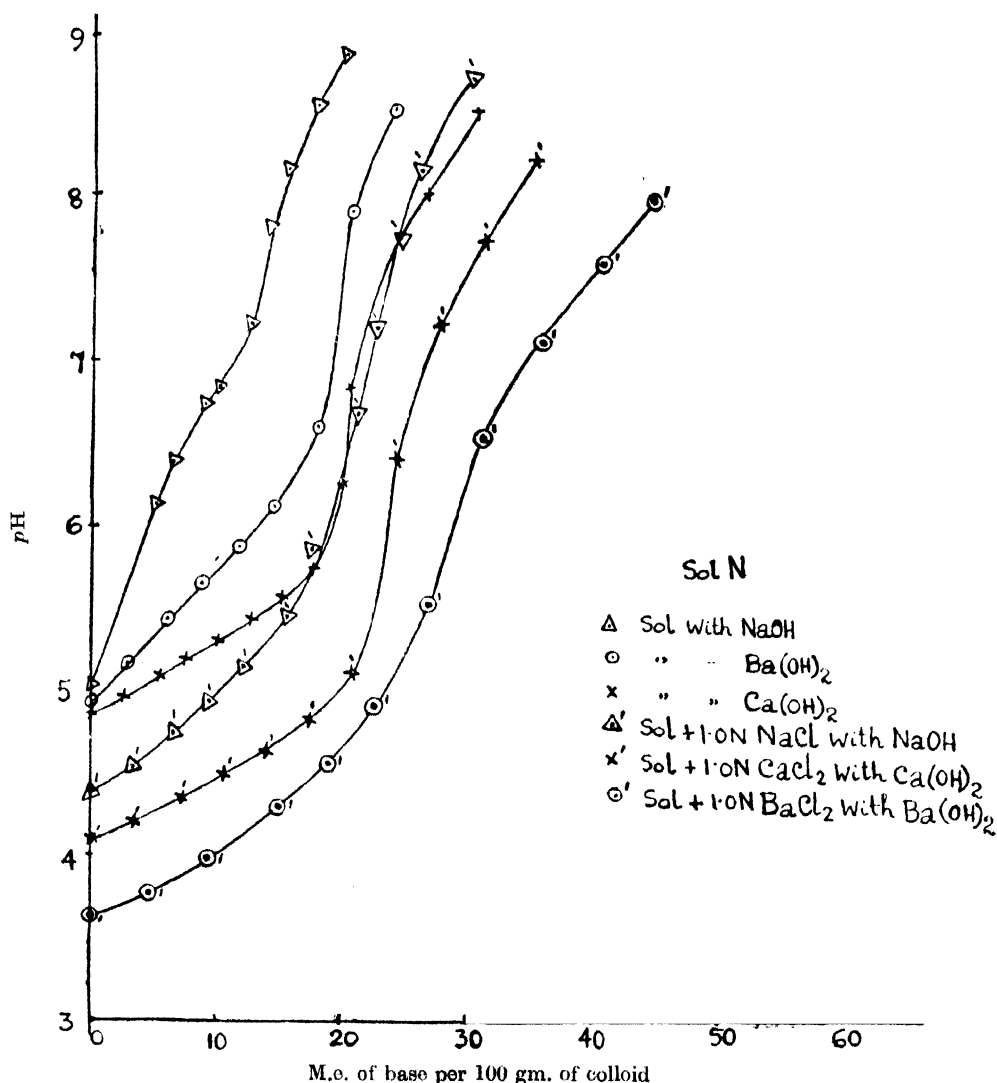


FIG. 9. Potentiometric titration curves of hydrogen clay N in the presence and absence of salts

A simple explanation of the cation effect and especially of its variation with the  $pH$  is not easy to offer in the light of the classical concepts of electrochemistry. The product of the interaction between hydrogen clay and mono and bivalent cations has often been regarded as an ordinary salt, the so-called

' clay salt ' and considerations of solubilities and degrees of dissociation of such clay salt have often been brought forward to explain the nature of such interactions and equilibrium conditions. Mattson [1932; 1935; 1937] has considerably developed this view. He postulates that, unlike ordinary salts, the clay salts are dissociated only to a limited extent and in the interaction  $H.AB.OH + MA' \text{ (or MOH)} \rightleftharpoons M.AB.OH + HA' \text{ (or HOH)}$  between the hydrogen clay  $H.AB.OH$  and the salt  $MA'$  or base  $MOH$ , the smaller the degree of dissociation of the clay salt  $M.AB.OH$ , the greater will be the displacement of the equilibrium to the right hand side of the equation. He postulates further that the salt ( $MA'$ ) suppresses the dissociation of the compound ( $M.AB.OH$ ) which the salt forms with the complex resulting in a further displacement of the equilibrium to the right hand side of the equation: the effect is similar to the suppression of the solubility of a salt by the addition of another having an ion in common. According to him, in the titration of the sol and salt mixture, therefore, ' a lot of anions is not produced to suppress the dissociation of the acid ' and consequently, compared with the sol itself the titration curve of the mixture gives a larger total acid at a fixed  $pH$ .

It has been previously shown that if the heterogeneous nature of the system is not taken into consideration, the concepts of the degree of dissociation and dissociation constant have little significance in the case of even such simple polyphase acid systems as saturated solutions of acids containing excess of the solid phase. The difficulties are more pronounced and differ even in character with colloidal solutions of acids, e.g. hydrogen clays. Further, solubility considerations have no real meaning with the so-called clay salt in the absence of any definite evidence to show that it forms a separate solid phase or an isomorphous mixture. The interaction has been shown to be limited to the surface. Finally, such considerations cannot reconcile the differences in the relative effects of cations, e.g. the Ba and Ca ions, in acid and alkaline regions as observed by us unless further *ad hoc* assumptions regarding changes in the relative solubilities of the barium and calcium ' clayates ' with the  $pH$  are made to fit the observations.

A consistent explanation of the cation effects and of the interactions of hydrogen clays in general has been previously given on the basis of the concepts of primary and secondary adsorption of which latter electrical adsorption forms a particular type as postulated by one of us [Mukherjee, 1921; 1922]. The primarily adsorbed anion is fixed on the surface, while the adsorbed cation is present on the liquid side of the double layer. The two form an ' ion pair ' which is fundamentally different from an ordinary salt molecule formed by the interaction between an acid and a base present either in the dissolved condition or in the solid state. The salt molecule has a definite solubility and it either remains in solution or separates out as a solid phase. The ion pair, however, is present in a peculiar phasal condition being present only on the surface. Besides a proportion of the cations neutralising the negative charge is present in the liquid and is thus in a dissolved condition, while the other part of the salt, the anion, is fixed on the solid surface and should thus be considered insoluble. Consequently, the ion pair as a whole can have no definite solubility in the usual sense.

The above picture has much in common with that suggested by Wiegner [1925; 1931]. The main difference consists in postulating the forces by which

the cations are held near the surface and, specially, the variation in the nature of these forces with the  $pH$ . Wiegner and Jenny [1927] also suggested the existence of electrical and specific forces of attraction to account for their observation that the alkaline earth metal cations are often adsorbed and released in different orders. They considered that adsorption was always brought about by electrical attraction. Once the cations were taken sufficiently near the surface by electrostatic attraction, they combined with the anion residues on the surface by more specific forces, e.g. chemical affinity, and the subsequent release of the cations from the resulting combinations depended on the nature and intensity of the binding force. Our work shows that adsorption itself may take place through electrostatic attraction as also more specific forces and, what is more important, the  $pH$  determines the nature of the force that operates.

The variations of free and total acids previously discussed point to the presence of  $H$  ions in different levels of reactivity (or affinity) on the surface. Of these, (1) the free, i.e. the osmotically active  $H$  ions present in the mobile sheets of the double layer are the most reactive and they give rise to the free acidity, that is the observed e.m.f. of the hydrogen or glass electrode. They are easily neutralized by  $OH$  ions and displaced by osmotic interchange with the cations of an added salt even if the latter are alkali metal cations and are present in small concentrations. In addition to these mobile  $H$  ions, there are (2)  $H$  ions secondarily adsorbed or bound which react with  $OH$  ions of the added base independent of the nature of its cations. In other words, in addition to the free  $H$  ions there are bound  $H$  ions on the surface which come out when the  $pH$  of the sol is increased. Lastly there are (3)  $H$  ions at a much higher affinity level which are released by strongly adsorbed cations whose nature and concentration determine the number of bound  $H$  ions set free at a comparatively low  $pH$ . The inflexion point in the titration curves with bases alone indicates in addition to the neutralization of  $H$  ions of the second category that of some  $H$  ions of the third category. A large number of these latter  $H$  ions are neutralized (at the inflexion point) when the sol is titrated in the presence of a large concentration of a neutral salt. A high concentration of a strongly adsorbed cation together with a high  $pH$  makes reactive the largest number of bound  $H$  ions. The limit to which the  $pH$  can be raised is conditioned by the stability of the adsorption complex. By making a suitable choice of the salt and its concentration it may be possible to reach a limiting value of the total acid, that is the b.e.c. Unpublished results of S. K. Mukherjee of this laboratory indicate the existence of such a limiting value. The b. e. c. given by Schofield's [1933] method approaches this limit.

#### *The acid character of hydrogen clay in relation to some problems of soil science*

An outline of the fundamental electrochemical character of hydrogen clay as an acid system has been given above. The manner in which it varies with some other properties of the hydrogen clay is indicated below. Details of these investigations will be published in separate series of papers.

#### *Variations in the properties of sub-fractions of hydrogen clay*

Sub-fractions of a hydrogen clay obtained by the graded centrifugalization of the entire clay fraction have been found to show the same broad features,

e.g. the regular and specific cation effects, as the entire hydrogen clay fraction. The base-exchange capacity (b.e.c.) of the different fractions usually increases with diminishing particle size as observed by other workers [Marshall, 1935]. However, the b.e.c. calculated per sq. cm. of the external surface\* does not usually remain constant, showing that the reaction with the base is not confined to the outer surface. The difference may also arise from the observed differences in the mass chemical compositions of the different fractions. The variations in chemical composition may arise from : (a) varying admixtures of free silica and sesquioxides in the different fractions, (b) the presence of different clay minerals in the various fractions, and (c) isomorphous replacements within the lattice of the same mineral contained in the different fractions. (b) and (c) would probably give rise to some definite alterations in the forms of the titration curves of the different fractions. Actually, however, the titration curves usually have more or less the same form, showing that the various fractions have fundamentally the same mineral constituent. An interesting observation made with most of the sub-fractions is the fact that their b.e.c. usually increases with a decrease in the silica-sesquioxide ratio. A positive correlation between the b.e.c. and this ratio is, on the other hand, usually observed with entire clay fractions [Mattson, 1932].

Our subsequent work on the sub-fractions includes, in addition to the above electrochemical studies, investigations on their X-ray and optical properties and their mineralogical compositions.

#### *Alterations in the properties on the removal of 'free' silica and sesquioxides*

The hydrogen clays have been found to show the same general features e.g. the cation effects, both before and after removal of the free inorganic oxide contained in them. These features are thus characteristic of the exchange complex itself. Definite variations in the b.e.c. were often observed on the removal of the free oxides. Using Mattson's [1932] method for this purpose a decrease in the b.e.c. was always noted. Tamm's [1922] method gave rise to a decrease in the case of some hydrogen clays, while with others, an increase was observed. In no case, a decrease in the b.e.c. was observed using Drosdoff and Truog's [1935] method. The b.e.c. either increased or remained unaltered. An increase in the b.e.c. has always been observed using the method of Truog *et al.* [1936].

#### *The rôle of aluminium ions in the interactions of hydrogen clays*

The rôle of aluminium ions in the interactions of hydrogen clays and acid soils with neutral salts is a much discussed problem. Some workers [Page, 1926 ; Wilson, 1929] consider that the acid liberated by the salt dissolves Al ions from the adsorption complex, while others [Daikuhara, 1914 ; Kappen, 1916] hold that a direct exchange of Al ions for the cations of the added salt takes place. The following are the possible sources of the displaced Al ions ; (a) free  $\text{Al}_2\text{O}_3$  contained in the hydrogen clay which is dissolved by the acid liberated ; (b) Al ions forming the lattice of the mineral constituents of the clay ; and (c) Al ions present on the surface in a secondarily

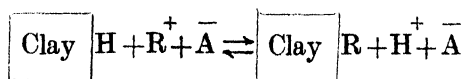
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\*In calculating the external surface, a spherical symmetry of the particles and a constant density of the different fractions were assumed.

adsorbed condition. Aluminium in all these three forms may react with acids, bases and salts. Toxic properties of acid soils are often attributed to the aluminium found in the soil solution [Comber, 1924]. It has been found [Mukherjee *et al.*, 1932 ; also unpublished work of Majumdar], however, that Al ions are stable on the surface of colloidal particles of aluminium oxide sols at a pH as high as 6.0. Fuller information regarding the part played by Al ions in the interactions of hydrogen clays under different conditions is therefore desirable.

It has been observed [Mukherjee and Chatterjee, 1942] that the titratable acidity of the neutral salt extracts of hydrogen clay is sometimes greater than can be accounted for by the amount of aluminium present in the extract when low concentrations (e.g. up to 0.002*N*) of the salt are used. The amount of aluminium increases with the concentration of the salt and at a sufficiently high concentration (usually near about 1.0*N*, using salts of the alkaline earth metal cations) it has been found to be almost equal to the titratable acidity. At very low concentrations, e.g. up to 0.002*N* using salts of the alkali metal cations, only hydrogen ions are displaced.

The hydrogen-ion activity increases on the addition of the salt and favours the back reaction in the simple schematic equation given below :



As the concentration of the salt increases, more and more Al ions are exchanged at the expense of H ions, but the two processes do not seem to be independent of each other although, as already stated, only H ions are exchanged at the lower concentrations of the salt. Experiments in which the pH was kept constant by the use of suitable buffers showed that the amount of aluminium liberated is the same as that when no buffer were used and the pH allowed to decrease as a result of the addition of the salt. The relation between the amount of Al liberated at a constant pH and the concentration of the added salt is given by a curve which closely resembles the adsorption isotherm. All these results indicate the presence of Al as well as H ions on the surface of the hydrogen clay particles. Ions of both categories are directly exchanged for the cations of the added salts.

### SUMMARY

The hydrogen clay is the inorganic part of the soil adsorption complex whose exchangeable cations have been replaced by H ions. It is usually made up of one or more secondary clay minerals, some comminuted primary minerals and 'free' oxides of Si, Al and Fe. The *ensemble* is an essentially electrochemical system—an electrolytic colloid with a dominant acid character. The main object of this investigation is the elucidation of this acid character.

Hydrosols of hydrogen clays are definitely polyphase acid systems, the insoluble acid material consisting of one or more phases and the intermicellar liquid another. While the sol has free acid usually of the order of  $10^{-4}$ *N*, its ultrafiltrate shows an almost neutral reaction.

The interpretation of the titration curves constitutes an important step in the elucidation of the acid character. With this idea, hydrogen clay has

been titrated under various conditions and the features of the titration curves have been carefully analysed. The titration curves of the following simple polyphase acid systems which are amenable to more straightforward theoretical treatment than the complex hydrogen clay have also been studied : (i) saturated solution of cinnamic acid containing the solid acid ; (ii) palmitic and stearic acid hydrosols ; and (iii) hydrosols of silicic acid.

The titration curves of cinnamic acid in the presence of the solid phase illustrate the difficulties in interpreting titration curves of heterogeneous acid systems. Kinetics of surface reactions play a definite rôle. The total amount of the acid taking part in the reaction is not constant but depends on the amounts of the alkali added. The course of the titration curve, however, can be fully understood knowing the solubility of the acid, its dissociation constant in true solution and the solubility of the resulting salt, sodium cinnamate. For the complex hydrogen clay the necessary information is lacking.

The titration curves of stearic acid sols with alkaline earth hydroxides illustrate features which would be expected if the hydrogen clay hydrosol were a heterogeneous acid which gives an insoluble salt like Ba- or Ca-stearate. Apart from some minor discrepancies the course of the titration curves of the stearic acid sol is fully accounted for by the phase rule.

Complexities are observed with silicic acid sols. The hydrogen ion activities, total acidities and the titration curves of the sols with bases differ in several essential respects from those of truly dissolved acids or from colloidal solutions of stearic acid. The total acidities at the first inflexion point are the same when titrated with sodium hydroxide as with barium or calcium hydroxide, but the slopes of the curves show a stronger adsorption of the alkaline earth metal ions. The first inflexion point occurs in the acid region between  $pH$  4.3 and 4.7 and would indicate, for acids in true solution, that a polybasic acid and/or a mixture of acids of different strengths are present in the solution. Analysis of the ultrafiltrate shows that dissolved acids cannot be held responsible for the course of the titration curve including the inflexion point in the acid region. The interaction with the alkali continues beyond this inflexion point. Further, when a barium or a calcium salt is added to the system an amount of neutralizable acid considerably larger than the total acidity at the first inflexion point is liberated. This inflexion point, therefore, in no sense corresponds to the neutralization of all the hydrogen ions capable of reaction and the degrees of dissociation or dissociation constants calculated on the assumption that the amount of acid neutralized at the inflexion point represents the total neutralizable acid present in the system, lose their usual significance. The hydrogen ions liberated by neutral salts pass into the ultrafiltrate but additional hydrogen ions are still present in the system. This is shown by the results of continued leaching. A definite cation effect associated with the energy of the formation of an ion pair by secondary adsorption is indicated. The complete titration curve of the sol with caustic soda shows on first examination that the sol appears to be a mixture of a moderately strong acid and a weak acid in true solution and that the latter is present in a very much greater concentration than the former. Calculations of the slopes of the conductometric and potentiometric curves and of the buffer indices show, however, that the resemblance is superficial.

Hydrogen clay sols present striking differences from acids in true solution. The total reacting acid or the base-exchange capacity (b.e.c.) of hydrogen clay is not a fixed quantity but depends on  $pH$  and cation effects. Usually, the greater the  $pH$  the greater is the b.e.c. The cation effect is illustrated by : (a) the dependence on the cation of the base of the b.e.c. calculated at the inflexion point and more strikingly at a fixed  $pH$ , e.g. 7.0 ; (b) by the much higher b.e.c. obtained on titration in the presence of a large concentration of a neutral salt than in its absence ; and (c) by the different effects of various neutral salts having a common anion. In the absence of salts the b.e.c. decreases in the order  $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{NaOH}$  which, however, changes to  $\text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2 > \text{NaOH}$  in the presence of a fixed concentration of the corresponding salts. The reversal in the relative effects of Ba and Ca ions has been traced to the differences in the  $pH$  region in which the acid-base reaction takes place. In the presence of the salt the interaction with the greater portion of the base  $\text{Ba}(\text{OH})_2$  or  $\text{Ca}(\text{OH})_2$  up to the inflexion point occurs in the acid region, usually between  $pH$  3.5 and 5.5 ; while in the absence of the salt it is mainly confined within the range of  $pH$  5.5-6.5. In the presence of salts the cation effect is regular in the sense that it follows the lyotrope series and is determined by the order of the electrical adsorption of the cations together with their hydration envelopes. At the comparatively high  $pH$  in the absence of salts the cations are probably adsorbed in a dehydrated condition, which accounts for the irregular or specific cation effect, irregular in the sense that it does not follow the lyotrope series, operating under these conditions. The regular and specific cation effects have been observed with sub-fractions of hydrogen clay having equivalent spherical diameters ranging between specified limits and separated from the same entire clay fraction and also with hydrogen clays after they have been treated by methods aiming at the removal of their free inorganic oxides.

The indefinite nature of the total neutralizable acid goes against the postulate of similarity with weak acids in true solution supported by some workers and invalidates the applicability of the concepts of degree of dissociation and dissociation constant in such systems. Hydrogen clay sols have low values, usually about 5-10 per cent, of the ratios of their free to total acids, that is, of the degree of dissociation in usual language. The potentiometric titration curves of the sols with the alkaline earth hydroxides have, on the other hand, usually a strong monobasic acid character. The inflexion points in the curves for these bases almost in all cases lie in the acid region. No second inflexion has been observed even on titrating up to  $pH$  12.

The cation effect also impresses itself on the form of titration curves. The curves with different bases have different forms. While the baryta and calcium hydroxide curves (potentiometric) have a flat initial run and thus have a strong acid character, the caustic soda curves show a comparatively sharp initial rise and in this respect resemble that of a weak acid. The features of the conductometric curves are at direct variance with those of the potentiometric curves. Thus the slopes of the descending portions of the conductometric curves are in the order  $\text{NaOH} > \text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2$ , indicating a stronger acid character of the NaOH curve compared with the  $\text{Ba}(\text{OH})_2$  or,  $\text{Ca}(\text{OH})_2$  curve.

Sub-fractions of the same hydrogen clay often show nearly the same type of titration curves. The b.e.c. per gramme generally increases with diminishing particle size. Calculated per sq. cm. of the external surface, however, the b.e.c. usually increases with the size of the particles.

When a neutral salt is added to a hydrogen clay sol, both H and Al ions are exchanged for the cations of the salt. It is only when salts of alkali metal cations are added in very low concentrations, e.g. up to 0.002N, that only H ions are exchanged. The relation between the amount of Al liberated at a constant pH and the concentration of the added salt is given by a curve which closely resembles the adsorption isotherm.

The conceptions of primary and secondary adsorption of ions, of electrical adsorption and adsorption by valence forces offer a satisfactory explanation of the results recorded in this paper. The interaction between the hydrogen clay and an electrolyte, apart from neutralization and other known chemical processes, involves exchange of H (and aluminium) ions present on the surface of the particles for the cations of the latter. The extent to which the cation will displace these H (and aluminium) ions will depend on the concentration of the added cation and the relative adsorbabilities of the ions. The larger b.e.c. of a hydrogen clay with alkaline earth hydroxides compared with the alkalis is thus to be attributed to the higher adsorbability of the alkaline earth cations compared with alkali metal cations. The adsorption of the cations is determined by their valencies, mobilities and states of hydration if adsorption is mainly the result of electrostatic forces. When it is brought about by chemical or valence forces, solubility and other considerations become of importance. The regular and specific cation effects, the differences in the slopes of the titration curves with different bases and features of conductometric and potentiometric titration curves, difficult of interpretation in the usual way, find a simple and consistent explanation.

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# \*STUDIES IN THE PERIODIC PARTIAL FAILURES OF THE PUNJAB-AMERICAN COTTONS IN THE PUNJAB

## V. PHYSICAL AND CHEMICAL PROPERTIES OF THE SOILS ASSOCIATED WITH *TIRAK* (BAD OPENING OF BOLLS)

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(With 1 text-figure)

THE idea of investigating the soil conditions in order to determine the causes of cotton failures arose as a result of observations made on the cotton crop during the cotton seasons of 1935-36 and 1936-37. It was observed that *tirak* or bad opening occurred in one part of the field, while normal plants were found in another part of the same field. It was also found later that *tirak* or bad opening occurred in the same field or in the same portion of a field whenever cotton was grown. These observations suggested that the soil factors might be associated with *tirak*. This view was also supported by the symptoms exhibited by the American cotton plants when they suffered from this physiological disease. Such fields were marked out in the cotton season of 1936-37 and soil samples, foot by foot up to a depth of 6 or 12 ft., were taken from these fields or portions of a field where the conditions of plants were found to be normal.

### INVESTIGATION

#### *Soils with sodium salts in the subsoil*

During the early stages of the soil investigation it was discovered that the soils where *tirak* appeared were alkaline varying from pH 9.2 to 9.8. The soils under normal plants were not so very alkaline in reaction, the pH fluctuating between 8.0 and 8.4. But such differences in the pH between the soils where normal and *tirak* plants were observed held good in some cases but not in others. The soil reaction was in the neighbourhood of pH 8.0 even when *tirak* was present (Table I).

Later it was discovered that though the subsoil under *tirak* was not alkaline, there were present abnormal amounts of total soluble solids in such soils indicating the presence of neutral sodium salts (Table II). This led to the determinations of total soluble solids in the soils from the normal and

\*The investigations described in this paper were carried out in the Punjab Physiological (Cotton Failures) Scheme financed jointly by the Indian Central Cotton Committee and the Punjab Government

*tirak* patches (Table II). A complete analysis of the soluble salts, foot by foot, showed that the soils where *tirak* appeared had a subsoil with abnormal amounts of soluble chlorides, sulphates and bicarbonates as compared with the quantities of these salts present in the subsoil where normal plants grew (Table III). The abnormal amounts of sulphates, chlorides and bicarbonates were present from either the third or the fourth foot downwards (Table III).

TABLE I

*pH of soils under normal and tirak plants at the Lyallpur Agricultural Farm*

Depth in ft.	Sq. 10		Sq. 27 D2		Sq. 27 D1		Sq. 26	
	Normal	<i>Tirak</i>	Normal	<i>Tirak</i>	Normal	<i>Tirak</i>	Normal	<i>Tirak</i>
1 . .	8.1	8.6	8.1	8.1	8.2	8.2	8.2	8.2
2 . .	8.2	9.6	8.0	7.9	8.1	8.1	8.0	8.3
3 . .	8.2	9.7	7.8	8.1	8.2	8.0	8.2	8.1
4 . .	8.3	9.7	8.0	8.7	8.3	8.0	8.1	7.9
5 . .	8.4	9.7	8.1	9.4	8.7	8.0	8.1	8.1
6 . .	8.4	9.6	8.1	9.6	8.8	7.9	8.1	8.3

In some cases there were also present small amounts of sodium carbonate, while it was absent in other cases. This explained the fact that the subsoils under *tirak* plants were more alkaline in reaction in some cases than the subsoils under normal plants, but this difference did not hold good in other cases.

The mechanical analysis of the soils under two types of plants showed that the sand fraction decreased while the clay and silt fractions increased in the subsoil of *tirak* patches, while that was not the case with the subsoil of the normal patches. This difference did not hold good in all cases, as for instance in the case of light sandy soils which are saline in the subsoil. When the soil samples up to 12 ft. depth were analysed it was sometimes found that the sand was at a lower depth in the case of normal soils than in the case of soils with salts in the subsoils (Table II).

TABLE II  
*Physical and chemical properties of soils under normal and tirak plants*

Depth in feet	Normal (sandy loam)					Tirak (sandy loam)					Normal (light sandy)					Tirak (sandy loam)				
	Clay (per cent)	Silt (per cent)	Sand (per cent)	Total solids (per cent)	pH	Clay (per cent)	Silt (per cent)	Sand (per cent)	Total solids (per cent)	pH	Clay (per cent)	Silt (per cent)	Sand (per cent)	Total solids (per cent)	pH	Clay (per cent)	Silt (per cent)	Sand (per cent)	Total solids (per cent)	pH
1	16	20	63	0.064	8.3	23	21	53	0.053	8.3	15	15	68	0.043	8.5	19	22	54	0.059	8.3
2	18	22	59	0.048	8.4	27	22	48	0.053	8.3	15	15	66	0.037	8.4	27	20	52	0.058	8.5
3	19	21	58	0.047	8.4	26	24	47	0.206	8.1	15	18	65	0.041	8.3	30	27	43	0.132	8.7
4	19	22	57	0.043	8.3	26	28	42	0.332	8.1	16	19	61	0.040	8.4	32	28	39	0.315	8.3
5	19	25	50	0.056	8.4	26	24	41	0.250	8.5	15	23	54	0.046	8.3	35	28	36	0.363	8.4
6	21	30	36	0.065	8.3	26	28	35	0.241	8.2	11	21	59	0.036	8.4	34	30	35	0.517	8.7
7	18	37	32	0.084	8.3	28	33	25	0.630	8.3	8	11	77	0.029	8.2	40	25	30	1.157	9.1
8	15	28	49	0.050	8.6	22	44	22	0.552	8.6	5	8	84	0.026	8.3	39	30	21	0.756	9.1
9	7	8	82	0.045	8.7	19	46	22	0.492	8.6	5	8	85	0.027	8.2	32	30	28	0.434	9.0
10	6	6	85	0.047	8.8	11	24	59	0.215	8.0	5	8	83	0.025	8.3	35	35	20	0.424	9.0
11	4	4	89	0.043	8.8	5	5	87	0.061	8.5	4	6	85	0.031	8.2	40	35	12	0.402	9.0
12	4	4	90	0.022	8.8	5	6	89	0.073	8.5	4	8	83	0.030	8.2	27	41	21	0.386	8.8

TABLE III

*Quantities of soluble carbonates, bicarbonates, sulphates and chlorides in gm. in 100 gm. of soil (calculated as sodium salts) in normal and tirak patches*

Depth in ft.	Normal					Tirak				
	Total soluble salts	Carbo- nates	Bicar- bonates	Chlo- rides	Sulphates	Total soluble salts	Carbo- nates	Bicar- bonates	Chlo- rides	Sul- phates
1st	0.084	0.003	0.089	0.001	0.0048	0.115	Traces	0.101	0.004	0.012
2nd	0.087	0.002	0.081	0.001	Nil	0.116	Do.	0.090	0.001	0.024
3rd	0.084	0.001	0.080	Nil	Nil	0.121	0.002	0.100	0.003	0.021
4th	0.078	Traces	0.061	0.003	0.010	0.215	0.002	0.189	0.023	0.055
5th	0.082	„	0.072	0.005	0.010	0.345	0.012	0.182	0.047	0.112
6th	0.078	„	0.066	0.004	0.011	0.448	0.023	0.202	0.076	0.154
1st	0.043	Nil	0.035	Nil	Nil	0.073	Nil	0.050	0.007	0.015
2nd	0.037	„	0.034	„	„	0.105	„	0.053	0.018	0.024
3rd	0.041	„	0.035	0.001	Traces	0.197	„	0.0884	0.051	0.075
4th	0.040	„	0.034	0.002	Do.	0.342	„	0.079	0.081	0.191
5th	0.046	„	0.037	0.003	Do.	0.442	„	0.065	0.079	0.274
6th	0.036	„	0.034	0.005	Do.	0.330	„	0.064	0.064	0.189

Pits were dug on *tirak* and normal patches of a field and the soil of each foot was separately collected up to a depth of 6 feet and was placed in six separate pots. Thus each of the six pots contained soil from any one-foot layer of the six one-foot layers of *tirak* patch. Similarly another six pots were filled with soil from any one-foot layer of the six one-foot layers from the normal patch. Seeds were sown in June 1936 in each of these pots. It was then found that either the seeds did not germinate or they did not grow further after the seedling stage in the pots which contained soils from either the 4th, the 5th or the 6th foot of the *tirak* patch, while the plants grew normally in pots containing soil from the 1st and the 2nd foot. The growth was subnormal in the 3rd foot of the soil. On the other hand, the plants grew normally in all the six pots containing soil from normal patch. The abnormal concentrations of the sodium salts proved toxic to the roots in pots containing subsoil layers of the *tirak* patch. This was not unusual, as it was later realized that similar thing occurred in fields where alkali salts, or *kalar* as is commonly called in the Punjab, was present on the surface. This fact was further confirmed by taking soil samples from a field under cotton where there was a big area irregular in outline, where 'no cotton plants' were present due to failure of germination. Surrounding this area there was a zone of stunted and scattered plants. Farther away from the second zone the field had a normal stand of cotton with medium growth. The soil samples up to a depth of 6 ft. were taken from each of these zones and analysed (Table IV). The result revealed the same features discussed above. In the area of no-plants the alkali salts were abnormally high from the 1st foot while they were less in amounts in the zone of stunted plants. They were still less in the region of the normal

TABLE IV  
*Properties of soils taken from regions of medium and stunted growth and no-plant region*

Depth in ft.	Medium growth of plants				Stunted growth of plants				Region devoid of plants						
	Clay (per cent)	Ssilt (per cent)	Sand (per cent)	Total soluble solids (per cent)	pH	Clay (per cent)	Silt (per cent)	Sand (per cent)	Total soluble solids (per cent)	pH	Clay (per cent)	Silt (per cent)	Sand (per cent)	Total soluble solids (per cent)	pH
1	14	23	51	0.109	8.1	18	22	51	0.318	8.3	17	26	51	0.612	8.2
2	10	23	54	0.103	8.1	23	26	43	0.498	8.3	20	27	44	0.708	8.4
3	6.1	29	54	0.088	8.2	24	25	36	0.768	8.2	20	32	39	0.759	8.4
4	3.6	9	84	0.067	8.3	26	33	26	0.742	8.7	22	35	39	0.850	8.5
5	0.8	12	84	0.071	8.2	26	42	23	0.708	8.7	22	46	17	0.804	8.4
6	0.7	9	88	0.050	8.2	20	37	31	0.576	8.7	21	44	20	0.840	8.4

stand of crop of medium growth. The mechanical analysis of the soils showed that the subsoil was sandy under normal stand of crop and heavy in the zones of scattered plants and devoid of plants.

It may be here pointed out that the soils of the Punjab are known to be very heterogeneous and the physical and chemical properties of the soils differ widely even in the same field measuring an acre. It is also found that the concentrations of alkali salts varied greatly in soils taken from two spots situated at a distance of a few feet (Table V).

TABLE V

*Percentage of soluble salts under normal and tirak plants found in the same field*

Depth in ft.	Lyalpur						Sargodha		Montgomery	
	Normal	Tirak	Normal	Tirak	Normal	Tirak	Normal	Tirak	Normal	Tirak
1 . . .	0.077	0.105	0.051	0.076	0.084	0.115	0.055	0.064	0.078	0.078
2 . . .	0.079	0.108	0.045	0.078	0.087	0.116	0.063	0.050	0.065	0.098
3 . . .	0.088	0.219	0.051	0.629	0.084	0.121	0.068	0.147	0.078	0.192
4 . . .	0.069	0.330	0.052	0.591	0.078	0.215	0.059	0.234	0.086	0.198
5 . . .	0.057	0.380	0.045	0.382	0.082	0.345	0.067	0.364	0.078	0.456
6 . . .	0.074	0.390	0.028	0.148	0.078	0.448	0.067	0.242	0.065	0.420

An idea of soil heterogeneity could be obtained from Fig. 1. A field measuring about one acre was selected for an intensive study of the soil conditions as *tirak* was found to occur in patches in 1936. The patches of *tirak* plants and normal plants were found irregularly distributed over the whole acre. The field was therefore divided into 48 small plots of 1/80 acre each and the soil samples were collected from these plots up to a depth of 6 feet. The soil samples were analysed for total soluble salts, physical structure, pH and soluble calcium and sodium. Cotton was sown in these plots in 1938 season and detailed observations on the condition of the crop were made throughout the season. These observations on the growth of the crop will be discussed elsewhere. It was found that normal and *tirak* conditions of the crop in each plot were associated with normal and abnormal concentrations of sodium salts in the subsoil. Fig. 1 has been prepared on the basis of normal and *tirak* condition of the crop as actually observed (unsown interstrips between the plots are not shown). It was sometimes found that normal plants occurred in one portion of the plot, while *tirak* plants occurred in another part of the same plot. So, fresh soil samples were collected to see the condition of the soil under each type of crop and the above-mentioned relationship was found to exist. When *tirak* crop is indicated in a plot in Fig. 1, it does not mean that the whole crop was *tirak* in the entire plot in all cases. There may be normal plants in some of these plots but the majority of plants had shown *tirak*. The intensity and spread of *tirak* was also found to vary in these plots in different years. In 1938 cotton season, the *tirak* appeared in a less intense form and was on a smaller area than in 1939. This difference will be explained later.

the conversion of calcium clay to sodium clay in the subsoil of the *tirak* patches had occurred, while it was not the case in other *tirak* patches, even though soluble sodium was found to be in higher amounts than soluble calcium (Table VIII). In the case of normal soils exchangeable sodium was nil or negligible and soluble salts were mostly calcium salts. It may be pointed out that in the following results exchangeable sodium plus potassium are given but separate determination of exchangeable potassium revealed that it always fluctuated between 0.2 and 0.5 milli-equivalents per 100 gm. of soils in normal as well as in *tirak* patches. So, when the value of exchangeable sodium and potassium is higher than 0.5 m.e., it must be taken that the remaining quantity represents exchangeable sodium alone.

TABLE VIII

*Exchangeable sodium+potassium and calcium in normal and tirak patches*  
(M. e. per 100 gm. of soil)

Depth in ft.	<i>Tirak</i>		Normal		<i>Tirak</i>		Normal	
	Exchange- able Na + K	Exchange- able Ca	Exchange- able Na + K	Exchange- able Ca	Exchange- able Na + K	Exchange- able Ca	Exchange- able Na + K	Exchange- able Ca
1st . . . .	0.99	7.4	0.56	5.8	1.4	7.8	0.9	7.4
2nd . . . .	0.78	8.6	0.25	7.0	2.4	8.4	0.9	8.2
3rd . . . .	0.43	8.6	0.26	6.8	3.4	6.6	0.6	9.2
4th . . . .	0.43	8.0	0.30	8.0	2.4	8.0	1.0	9.4
5th . . . .	1.17	8.4	0.30	6.6	5.4	6.4	0.4	9.0
6th . . . .	0.78	8.6	0.30	7.1	5.4	6.2	0.6	7.2

The degree of sodiumization of clay, i.e. the amount of exchangeable sodium, was not found to bear any relation to the total soluble sodium salts present. In many cases even though the total soluble sodium salts were very high the exchangeable sodium in the clay complex was low, while the quantity of exchangeable sodium was high in soils containing lesser amounts of soluble sodium salts (Table IX).

Thus, the soils where *tirak* appears are found to contain abnormal amounts of total soluble solids in the subsoil from the 3rd or the 4th foot downwards. The soluble solids contain larger quantities of free sodium than calcium. In some cases sodium has replaced calcium in the clay complex, while in other cases similar base exchange has not taken place even though free sodium is found to be in excess of calcium. Except for the above-mentioned differences, the soils from *tirak* patches were not found to differ in any other character.

A large number of results collected from soils of different fields in different districts where *tirak* was noticed are available in support of the above conclusions.

TABLE IX

*Soluble and exchangeable sodium and calcium in tirak and normal soils\**

Depth in ft.	Sargodha					Lyallpur				
	Total solids (per cent)	Soluble Na (per cent)	Soluble Ca (per cent)	Ex-change-able Na + K (m.e.)	Ex-change-able Ca (m.e.)	Total solids (per cent)	Soluble Na (per cent)	Soluble Ca (per cent)	Ex-change-able Na + K (m.e.)	Ex-change-able Ca (m.e.)
(a) Tirak patches										
1st . . .	0.063	0.003	0.008	1.05	9.4	0.037	0.003	0.008	0.9	9.8
2nd . . .	0.058	0.008	0.007	0.88	11.2	0.076	0.029	0.006	1.1	11.6
3rd . . .	0.140	0.014	0.005	2.12	9.2	0.497	0.148	0.047	Nil	11.2
4th . . .	0.235	0.059	0.004	3.00	7.21	0.583	0.155	0.039	„	9.2
5th . . .	0.304	0.062	0.008	2.12	6.8	0.581	0.167	0.029	„	7.8
6th . . .	0.242	0.080	0.005	1.35	6.0	0.375	0.148	0.013	4.2	5.6
(b) Normal patches										
Sargodha					Montgomery					
1st . . .	0.055	0.003	0.014	0.60	10.6	0.080	0.003	0.010	0.8	6.6
2nd . . .	0.063	0.003	0.012	0.48	12.4	0.098	0.002	0.009	0.8	7.0
3rd . . .	0.068	0.003	0.015	0.54	13.4	0.086	0.001	0.008	0.6	7.6
4th . . .	0.058	0.003	0.013	0.65	12.6	0.094	0.002	0.009	0.4	8.4
5th . . .	0.067	0.004	0.015	0.48	12.6	0.098	0.001	0.008	0.6	8.4
6th . . .	0.067	0.003	0.013	0.43	10.3	0.094	0.001	0.008	0.6	7.2

\*Exchangeable potassium is generally 0.2—0.5 m.e.

It was pointed out above that out of three bores taken on a *tirak* patch one bore showed abnormal amounts of total salts, while the remaining two bores contained slightly more than the normal quantities of these salts. The total soluble salts were found to vary from 0.1 to 0.15 per cent in all the layers of the soil. When these soil samples were further analysed, it was found that, even though the total salts were not abnormally high, there were present larger amounts of soluble sodium than calcium (Table X).

TABLE X

*Soluble sodium and calcium in soils with medium and low salinity in the subsoil (per cent)*

Depth ft.	Total soluble salts	Soluble sodium	Soluble calcium	Total soluble salts	Soluble sodium	Soluble calcium
1st . . .	0.079	0.003	0.007	0.078	0.003	0.007
2nd . . .	0.073	0.003	0.007	0.066	0.003	0.006
3rd . . .	0.074	0.004	0.008	0.054	0.004	0.005
4th . . .	0.095	0.010	0.006	0.060	0.008	0.004
5th . . .	0.110	0.016	0.008	0.060	0.010	0.006
6th . . .	0.106	0.018	0.007	0.072	0.013	0.006

Thus, it was found that in between the soils which had a high and abnormal salinity in the subsoil, soils with low or medium salinity were also present. The term salinity is used here and hereafter to indicate that the subsoil contained more of soluble sodium salts than calcium salts or more of exchangeable sodium than exchangeable calcium, i.e. for soils which have a low sodium/calcium ratio as contrasted with a high sodium/calcium ratio in normal soils (without salinity). In a *tirak* patch one may not come across a subsoil which is highly saline over the whole area and some bores may show a subsoil which has a low salinity or no salinity at all. The cotton plants that were seen on such fields or patches were affected in their growth and opening of the bolls according to the nature of the subsoil. It was found that *tirak* and normal plants occurred irregularly distributed in such fields or patches whenever cotton was grown. It was also found that the total area under *tirak* plants increased in such fields or patches during years of unfavourable weather conditions. The *tirak* in such years was found to spread to soils where the subsoil was of a medium salinity. *Tirak* was found to be present in parts of a field, in the cotton season of 1939, where it was not observed before. An entire field may have subsoil with a medium or low salinity and in such fields *tirak* appeared in some years but in other years the crop was found to be normal. On such soils the equilibrium of the crop with the soil could be upset by warm and dry weather or by inadequate water supply. Any one of these factors can tip the scales off the normal condition and *tirak* condition would result.

The salt tolerance of a plant is known to be affected by temperature. Ahi and Powers [1938] have shown that salt tolerance of certain grasses and legumes increased at low temperatures and decreased at high temperatures. It is therefore probable that when the temperatures are high the salt tolerance is decreased and the subsoil of a medium or low salinity may during that period affect adversely the root systems of the cotton plants and cause *tirak* on such soils.

Joseph [1925] working on the soils in Sudan has also reported the presence of high concentrations of alkali salts in the 3rd and the 4th foot. The concentration of alkali salts which according to him consisted mostly of sulphates and carbonates in these layers was about 0.3-0.4 per cent; while in the 1st and the 2nd foot it was less than 0.1 per cent. The main differences between the soils in Sudan and the soils in the Punjab are in their clay contents, the alkalinity and chlorides. The clay content in Sudan soils is about 55 per cent and the pH is about 9.3. The chlorides, on the other hand, are lower in the Sudan than in the Punjab soils. The same author tried to show that salt contents were inversely related to yields, but an examination of his results did not justify that conclusion. Some of his high-yield plots showed high percentage of salts, while some low-yield plots showed low percentage of salts. It is therefore likely that the soils in Sudan are as heterogeneous as they are here and soils with low and high salinity are intermixed as they are in the Punjab.

The development of *tirak* on soils with saline subsoil was also found to depend on other soil conditions, the most important being the physical texture of the soil. A medium salinity in the subsoil was enough to produce *tirak* on lands which were light sandy, while *tirak* did not occur under similar condition

if the soil was sandy loam with a higher percentage of clay and silt except under abnormal conditions of weather or water supply. High salinity in the subsoil in a light sandy soil produced the worst form of *tirak* as the toxic effect of sodium on the roots was very high under such conditions. In light sandy soils smaller amounts of sodium either in the soluble or exchangeable form was found to produce a *tirak* crop, while in heavier types of soils *tirak* did not occur under similar conditions.

Similar observations have also been made by Harris [1920], Headley, Curtis and Scofield [1916], Harris and Pittman [1918] and Kearney and Scofield [1936]. Kearney and Scofield [1936] found 0.2 per cent of total salts toxic to alfalfa in sandy soils, while larger amounts were needed to produce toxic effects in loamy soils.

The toxic effect of sodium salts would also depend on the nature of the sodium salt present. Generally sulphates, chlorides and bicarbonates of sodium were found to be present, but the relative proportion of each was found to vary in different soils. The chlorides are known to be more toxic in their effects than sulphates and bicarbonates, and therefore the adverse effect on the crop produced by these salts would depend on the relative amounts of these salts present and their total quantity.

There is a definite indication in the work done on the effect of alkali salts on the growth of plants that chlorides are more toxic than sulphates or bicarbonates. It is pointed out by Harris [1915] that the acid radicals, i.e. the anions, determine the toxicity of alkali salts and not the cations, and the chloride ion has been found to be most toxic amongst anions, while the sodium was the most toxic amongst cations. He has shown that toxicity of sodium chloride was highest amongst the soluble salts in the soil. Voeleker [1916] has also found that sodium chloride was most toxic if present in concentrations of 0.2 per cent, i.e.  $2\frac{1}{2}$  tons per acre.

It is also known that limit for toxicity for different sodium salts for a plant is different. Hilgard [1906] has determined the range at which each sodium salt proves toxic. They are 0.1-0.25 per cent for carbonate 0.3-0.5 per cent for chloride and 0.5-1.0 per cent for sulphate. Hibbard [1906] has shown that much smaller amounts than those may prove injurious to some plants, but there is an agreement that sodium sulphate and sodium bicarbonate are less injurious than sodium chloride and sodium carbonate.

The analysis of the water extract from the *tirak* patches showed that chlorides were always high in the subsoil of *tirak* patches, while they were always very low in the subsoil from normal patches. In normal soils the soluble salts consist mostly of bicarbonates which are salts of calcium and not of sodium. As sodium carbonate was generally absent in *tirak* soils, the toxic effect on the cotton plant might be due mainly to sodium chloride as sodium sulphate and bicarbonate were known to be the least toxic sodium salts. It will be shown in a later contribution that sodium chloride has been found to be more toxic to cotton than either sodium bicarbonate or sodium sulphate.

The presence of salinity in the subsoil was found to depend under irrigated conditions on the depth of the soil crust, i.e. on the depth at which sand layer was present. In some cases it was noticed that, while the upper 6 feet contained normal amounts of total soluble salts, high concentrations of total soluble salts were found to be present at greater depths than 6 feet. The mechanical

analysis of the soil revealed that the percentage of sand increased from the 7th foot downwards. It appeared that though soluble sodium salts were present in the upper soil crust, irrigations washed them down gradually to lower layers of sand. The presence of sand prevented the salts from rising up again and the salts got gradually washed down to the sand layer. The analysis of such a soil is given in Table XI.

TABLE XI

*Field showing the accumulation of salinity at the lower sandy depths*

Depth in ft.	Sand per cent	Total soluble solids per cent (gm.)	Soluble Na (mg. per 100 gm.)	Soluble Ca (mg. per 100 gm.)	Exchangeable Na + K in m.e.	Exchangeable Ca in m.e.
1st . .	50	0.0471	11.1	7.5	2.4	8.0
2nd . .	48	0.0455	9.4	7.1	1.8	9.0
3rd . .	44	0.0511	8.1	7.9	1.6	7.4
4th . .	36	0.0550	12.0	5.0	1.4	5.4
5th . .	46	0.0791	12.0	5.4	1.8	2.8
6th . .	54	0.0621	11.3	4.6	0.4	2.8
7th . .	63	0.1296	24.5	4.0	0.6	2.8
8th . .	68	0.1516	33.2	4.8	0.8	3.4
9th . .	80	0.1601	36.5	6.2	0.7	4.4
10th . .	89	0.1145	19.6	4.5	0.5	3.2
11th . .	87	0.0796	11.8	3.4	0.4	2.4

The results showed that the percentage of sand rose to 63 in the 7th foot and it began increasing downwards from that layer. The total soluble salts were higher in the lower layers of sand than in the upper layers of the soil crust. Even though the total soluble salts in the soil crust were normal, soluble sodium was present in larger amounts than calcium in these upper layers of the soil. The ratio of exchangeable sodium to calcium was also low indicating that while the salts were washed down base exchange had occurred and sodium clay was produced. Worst form of *tirak* was observed in this field in 1938. The soil samples were collected from different spots in this area and one bore showed high concentrations of the soluble salts in the first 6 feet, indicating that sodium salts were not washed down by irrigations in some portions of the field.

It should be clear from above that the relations between salinity in the subsoil and *tirak* were complex and a study of the crop was necessary along with the study of the soil conditions. This relationship between salinity and *tirak* was correctly visualized after a great amount of detailed work both in the laboratory and the field. The heterogeneity of the soil in the same portion of a

*tirak* patch had added to the difficulty as all the bores from a patch did not indicate the same degree of salinity in the subsoil. It is therefore necessary that soil samples must be taken while the crop is standing, otherwise very great confusion may arise on account of the heterogeneity of the soil.

#### SOILS WITH NITROGEN DEFICIENCY

The soils where the crops showed symptoms of nitrogen starvation and poor opening of the bolls were found to be light sandy soils containing a large proportion of sand varying from 55 to 70 per cent. The symptoms exhibited by the plants that suffered from *tirak* on soils with saline subsoils resembled in many ways the symptoms that developed in the plants that suffered from lack of nitrogen at the flowering stage and crops which suffered from *tirak* due to nitrogen deficiency could not be distinguished in the early stages of this investigation from the crops which suffered from *tirak* on soils with saline subsoil. It was also found that in some fields crops suffered from *tirak* due to both the causes.

The soils where nitrogen deficiency occurs are normal soils containing normal amounts of soluble salts which are mostly salts of calcium, while the amount of sodium either in soluble or in exchangeable form is nil or negligible (Table XII). The plants make normal growth till August when the leaves begin to turn yellow and are shed. The bolls are few and small containing small seeds. Generally the seeds are lighter in weight than normal. Application of sulphate of ammonia to such soils was found to remedy *tirak*. The relation between nitrogen deficiency and bad opening of the bolls has already been described in a previous contribution [Dastur, 1941].

TABLE XII

*Physical and chemical properties of light sandy soils with nitrogen deficiency*  
(Per 100 gm. of soil)

Depth in ft.	Clay (per cent)	Silt (per cent)	Sand (per cent)	Total soluble salts (per cent)	Exchange- able Na+ K (m.e.)	Exchange- able Ca (m.e.)	Sol. Na (per cent)	Sol. Ca (per cent)
1st	11	13	76	0.077	0.8	6.8	0.002	0.006
2nd	13	18	65	0.066	0.4	8.6	Nil	0.006
3rd	14	20	62	0.060	0.8	8.6	Nil	0.007
4th	15	24	63	0.080	0.6	8.0	Nil	0.008
5th	13	21	58	0.060	0.4	5.0	0.001	0.007
6th	10	19	62	0.060	Nil	3.8	0.002	0.006

It was found that fields with such light sandy soils deficient in nitrogen contained patches which had sodium clay in the subsoil. In some cases the soluble salts though normal in quantities contained more of sodium salts than of calcium salts. Such patches of saline subsoil were found irregularly scattered about in light sandy fields with a deficiency of nitrogen. At one place a big or a small patch was found to contain sodium clay or higher amounts of soluble sodium salts in the subsoil, while in soil surrounding such a patch may have normal subsoil. Such intermingling of normal soil (light sandy) with soil with sodium clay in the subsoil or with abnormal amounts of sodium salts in the subsoil within a small area was found to occur. The detailed study of the crop, the analysis of the soil underneath, the effect of the application of ammonium sulphate on the plants' growth, the chemical analysis of the leaves and the tannin test on the leaves disclosed the nature of the soil conditions that were associated with *tirak* due to salinity in one case and due to nitrogen starvation in the other. The sulphate of ammonia was found to produce beneficial effect on the vegetative growth of the plants but was not found to have ameliorative effect on the opening of the bolls when the subsoil contained sodium clay or sodium salts in larger proportions than calcium salts.

From what has been stated above it is necessary to distinguish *tirak* or bad opening that occurs on soils with saline subsoil from *tirak* caused by the deficiency of nitrogen. It is now possible, by examining the crop in the fruiting stage, to know pretty exactly if the symptoms of *tirak* were caused by a deficiency of nitrogen in the soil or not. The application of tannin test was another rapid method of knowing the deficiency of nitrogen as explained in a previous contribution [Dastur, 1941]. Similarly high concentrations of sodium salts in the subsoil could be known by the drooping leaves of the crop on such soils. The drooping of the leaves occurred a week after irrigation, and in such cases the leaves did not assume normal position in the evening or in the morning. The leaves turned black green and were gradually shed.

The term *tirak* which is in common use in the Punjab to denote the condition of the American cotton crop on the two soil types is retained here even though it is not scientifically correct to do so. There are some differences in the symptoms, as well as in the nature of physiological disturbances produced within the plants, under the two types of soils, but these soil conditions are so mixed up together even in small areas that it is deemed desirable to differentiate the two soil conditions without differentiating *tirak*.

#### DISCUSSION

It is quite clear from above that mere collection of soil samples and analysing them would not give any clue to the above established relationships. It is not enough to be told by a zemindar that *tirak* had occurred in such a field. On account of the great heterogeneity that existed in the soil, it was absolutely necessary to make sure of the exact spots where *tirak* had occurred. It was necessary to take a number of bores even after the spot was located as the salinity in the subsoil was very variable. Many a time crops which are inadequately irrigated also showed shedding of leaves and badly opened bolls. They should not be mistaken for *tirak* as it occurred under saline conditions. Lack of cultivation may also produce badly opened crop.

It was also noticed that light sandy soils deficient in nitrogen occurred at one end of a line of 2 acres, while soils with a very heavy and saline subsoil occurred at the other end. In one square of land (i.e. 25 acres) all types of soil conditions may be found. It is this intermingling of normal and *tirak* soils in a small area that had so far obscured the soil conditions associated with *tirak*. In a year of favourable season, the cotton crop in a field may show *tirak* only on patches which have a highly saline subsoil, while the crop may be normal in the remaining parts of the field. Consequently *tirak* is not readily noticeable though it is there. But *tirak* becomes noticeable when adverse weather conditions cause *tirak* to spread on the portions of the fields where a medium or low salinity exists in the subsoil. If the soil samples are taken in such a year, it is possible the analysis may only show low salinity in the subsoil and a conclusion may be drawn that there was nothing abnormal.

The heterogeneity of the soil conditions in a small area would render the practical application of any remedial measure to such soils a difficult task. As for instance, heavy irrigations can wash the salts downwards from the feeding zones of the roots but that remedy can only be applied if a square of land is found to be uniformly saline underneath. In the same square, normal land, light sandy land, land with low, medium and high salinity and with the presence of sand layer at varying depths may be found. Heavy irrigations or rice cultivation will therefore produce varying effects on the different soil types. They will wash down the salts at some places but they will also wash down the important nutrients at other places, rendering the soil infertile. This has actually happened on light sandy soils under normal conditions of cropping and irrigation. Similarly, the applications of sulphate of ammonia to a field may give varying responses, varying according to the nature of the subsoil. The crop would be benefitted at places where the soil is light and sandy and non-saline, but no benefit would be derived if the soil is sandy loam with saline subsoil. Sulphate of ammonia will ameliorate *tirak* on light sandy soils with a normal subsoil but will not do so in parts of the field where there are sodium salts in the subsoil.

The heterogeneity of soil renders difficult the task of studying the effects of various manurial treatments on cotton under field conditions. It has been the experience of the Agricultural Department that nitrogenous manures gave high responses in one year or in one field and low and no responses in another year or in another field. The reasons for such varying results obtained could now be understood.

The primary cause of this physiological disorder or disease named as *tirak* may be traced to sandy nature of the cotton tracts in the Punjab. The toxic effect exerted on the roots of cotton plants by the levels of concentrations of sodium salts given above would be much less if the clay contents were high. The sandy nature of the soil also promotes very good root growth and consequently shoot growth of the plant in the first three months of the plant's life, and they therefore later suffer either from nitrogen starvation or a water deficit. The experimental evidence to support this view will be described in another contribution on the subject.

It may be argued that the association of a certain soil condition like salinity with *tirak* would not mean that this soil condition was the cause of *tirak*. The following reasons are put forward to support the view that the presence

of salinity in the subsoil was causing the phenomenon of *tirak* in the American cotton plants :—

1. The toxic effects of sodium salts, especially sodium chloride and sodium carbonate, on the growth of plants both wild and cultivated are too well known.

2. Sodium salts were not found to be present in the subsoils where *tirak* did not occur under any condition of weather and under normal conditions of irrigation.

3. The cotton plants do not grow on soils where the alkali salts are present on the surface.

4. It has been possible to reproduce *tirak* as it occurred in nature by artificial applications of sodium salts to the soil where there was originally no salinity in the subsoil. These experiments on artificial reproduction of *tirak* in fields with normal lands will be described in another contribution.

5. It is now established that the plants on soils with a highly saline subsoil suffer from a disturbance in their water supply at the reproductive stage when the total leaf area has reached its maximum point. This disturbance in the water balance is found to be partially removed by heavy or frequent irrigations in September-October or by reducing the leaf area of the plant by taking recourse to sowing cottons a month later than the normal time, on such soils. These experiments will be described in another paper.

So far attempts have been made to establish the relation of salinity in the subsoil with the symptoms of *tirak* or bad opening of bolls in the American cotton plants as that has been the main object of this investigation. These findings, however, raise the important and fundamental question of the origin of salinity in the Punjab soils and of the causes that may be responsible for salinization to occur at one place and not at another place in the same field or in the same square. Nothing is known about this process of salinization as it has occurred in the Punjab soils. It is not understood whether salinization is an up-grade process or a down-grade process and whether the change is from the saline to the non-saline condition or from the non-saline to the saline condition. It is not known whether the sodium salts were present in the soil crust when it was formed or whether they have been produced by weathering or chemical changes brought about under irrigation. It is needless to emphasize the urgency and importance of this knowledge from academic as well as practical point of view.

### SUMMARY

*Tirak* or bad opening of the bolls in the Punjab-American cottons is found to occur on two types of soils and the physical and chemical properties of these soils have been investigated along with the properties of the soils where normal crop grows.

The soils where *tirak* occurs are found to contain abnormal amounts of sodium salts (0.2 per cent or more) in the subsoil from the 3rd or the 4th foot downwards. Sodium in the soluble or exchangeable form is found to be higher than calcium. Such soils are found located in an entire field or in portions of the field. Such *tirak* patches are found surrounded by normal

(non-saline) soils. *Tirak* is found to occur in such patches every time cotton is grown there.

If the quantity of total salts is not high but medium varying from 0.1 to 0.15 per cent, *tirak* does not appear under favourable conditions of weather and under adequate water supply, but it is developed on such spots in years of dry and warm weather or in absence of adequate water supply. The physical texture of the soil, the sodium/calcium ratio and the relative amounts of different sodium salts present are important soil factors that increase or decrease the intensity of *tirak*.

Another soil type where *tirak* occurs is the light sandy land which produces a deficiency of nitrogen in the plant at the flowering stage. These soils are otherwise normal and *tirak* can be ameliorated by the application of sulphate of ammonia [Dastur, 1941].

The soils with high, medium or low salinity in the subsoil are found intermingled in the same area. Adverse weather conditions in a certain year bring about a wider spread of *tirak* in a field in that year than in a year of normal or favourable weather.

The light sandy soils may contain normal (non-saline) subsoils or may also contain subsoils with sodium salts or with low sodium/calcium ratio in exchangeable form. If the soil is light sandy with salinity in the subsoil, *tirak* occurs in the most intense form.

All these soil types may be found in the same square (25 acres). One end of a field may have light sandy soil, while the other may have sandy loam with salinity in the soil. The relationship of these soil conditions with *tirak* were established from a study of (1) the growth of the crop in small plots, (2) results of detailed analysis of the soil underneath normal and *tirak* crops, (3) results of mineral analysis of the leaves, (4) the response to the application of sulphate of ammonia and (5) the tannin test.

Sandy loams with a saline subsoil did not respond to applications of sulphate of ammonia, while a light sandy soil without salinity gave a high response to this fertilizer.

Various suggestions are made regarding the methods adopted to establish this relationship between soil conditions and *tirak* for the guidance of future workers.

Experimental evidence to support the view that salinity in the soil is the cause of *tirak* on such soils will be published in another contribution.

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# EFFECT OF TEMPERATURE AND TIME ON DRY WEIGHT DETERMINATION OF MANGO PULP

BY

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(With two text-figures)

**D**ETERMINATION of total dry weight in fruits containing a large amount of both sugars and water is beset with considerable difficulties. It has been shown by Archbold [1925; 1928] that the usual method of estimating dry weight by drying at 100°C. does not give a constant weight for apple pulp even after prolonged heating, because at this temperature decomposition of sugar and liberation of volatile compounds are the possible sources of error in having a constant value. Further, it has been shown by her that the sum of sugar, acid and alcohol-insoluble material is greater than the total dry weight obtained, which suggests that this method of dry weight determination in apples is not satisfactory. She has suggested that drying in *vacuo* at room temperature for three weeks or drying at 50°C. at atmospheric pressure is best suited for routine work. But the temperature and the time of drying is required to be determined for the tissue and drying apparatus in use. In view of this the behaviour of dry weights of mango pulp with rise in temperature and with duration of heating was studied, and the results are presented in this paper.

Mangoes (variety Fazli) were collected from a single healthy tree in an orchard at Malda. After peeling of the skin, pulp from the middle portion of the mesocarp was cut into thin slices. The slices from different fruits were thoroughly mixed up and fixed in warm alcohol (95 per cent). Soxhlet extractor was then used for separating the alcohol soluble and insoluble materials. The initial dry weights of the alcohol insoluble residue were obtained by drying over phosphorus pentoxide to a constant weight at the laboratory temperature and of the alcohol soluble material by first removing alcohol in vacuum distillation and then drying it over phosphorus pentoxide. Then the materials were dried for 24 hours at each of the five different temperatures in succession, namely 31°C. (room temperature), and gas oven temperatures of 50°C., 70°C., 90°C., and 100°C. Next the effect of prolonging the time of drying at 50°C. was investigated. Initial dry weight of both alcohol soluble and insoluble portions were obtained by drying over phosphorus pentoxide as before, then the successive periods of 24 hours drying were done at 50°C.

**TABLE I**  
*Effect of temperature on dry weight*  
 (Results expressed as percentage of fresh weight)

Sample	Temperature (°C.)	Material		Difference	
		Alcohol-soluble	Alcohol-insoluble	Alcohol-soluble	Alcohol-insoluble
I Green mango	31 (room temperature)	9.770	2.320	0.517	0.009
	50	9.253	2.311	0.677	0.032
	70	8.676	2.279	1.110	0.017
	90	7.566	2.262	0.390	0.002
	100 (for 8 hours)	7.176	2.260		
II Ripe mango	31 (room temperature)	16.130	5.027	0.820	0.031
	50	15.310	4.996	0.600	0.113
	70	14.710	4.883	0.734	0.000
	90	13.976	4.883	0.540	0.003
	100	13.436	4.880		

Table I and Fig. 1 show that the dry weight of alcohol-soluble material markedly falls with rise in temperature in both green and ripe mangoes, while the loss in alcohol-insoluble material is insignificant. The rate of fall in alcohol-soluble material of the green mango rises with temperature except at 100°C. drying where the fall is comparatively small and this is due to the fact that drying at this temperature was continued only for eight hours and also difference in temperature was 10°C. instead of 20°C. With 24 hours drying at 100°C. we might expect a greater loss. The data presented in Table II and Fig. 2 show a parallel behaviour of two samples with different periods of drying. It will be evident that the total loss in alcohol-insoluble material at 50°C. drying for 120 hours is negligible. Hence drying at 50°C. for 24 hours at atmospheric pressure may be safely used for dry weight determination of alcohol-insoluble materials. In the case of alcohol-soluble material the loss after 24 hours' drying at 50°C. is greatest, and then a steady rate of loss is obtained till the total time of drying is 72 hours, after which the rate slows down. This steady rate of loss at this temperature is interesting to note. If simple dehydration was occurring, the rate of loss would continue

to diminish. It must be due to a reason other than dehydration. The same phenomenon has been noted by Archbold in apples, where a steady fall takes place from 36 to 130 hours drying. She suggested that the juice possibly contains a volatile constituent of high density, which was removed during the drying process. In a later year a volatile polyhydric alcohol, sorbitol, was isolated by Tutin [1925] in apples, and recently in pears by Kidd, West, Griffith and Potter [1940]. Since in mangoes the temperature effect in losing dry weight of alcohol soluble material has been noted, an attempt was made to isolate sorbitol in the form of an insoluble benzal compound by a method suggested by Kidd, West, Griffith and Potter. Although sorbitol was not detected, the presence of some other polyhydric alcohol was presumed. The nature of this substance is not known, but attempts are being made to isolate it. An indirect evidence of the presence of it is the highly hygroscopic nature of the alcohol-soluble substance. While weighing even after drying at ordinary temperature the alcohol-soluble material was found to absorb water readily and the higher the temperature used for drying the greater was its hygroscopic nature.

TABLE II  
*Effect of time of heating on the loss of dry weight*  
(Results expressed as percentage of fresh weight)

Sample	Temperature (°C.)	Time of drying in hrs.	Material		Difference	
			Alcohol- soluble	Alcohol- insoluble	Alcohol- soluble	Alcohol- insoluble
III	31 (room tem- perature)	..	18.90	2.164	0.37	0.024
		1—24	18.53	2.140	0.11	0.008
	50	24—48	18.42	2.132	0.11	0.000
		48—72	18.31	2.132	0.07	0.002
		72—96	18.24	2.130	0.03	0.000
		96—120	18.21	2.130		
IV	31 (room tem- perature)	..	16.65	1.750	0.39	0.006
		1—24	16.26	1.744	0.11	0.004
	50	24—48	16.15	1.740	0.11	0.000
		48—72	16.04	1.740	0.05	0.001
		72—96	15.99	1.739	0.03	0.000
		96—120	15.96	1.739		

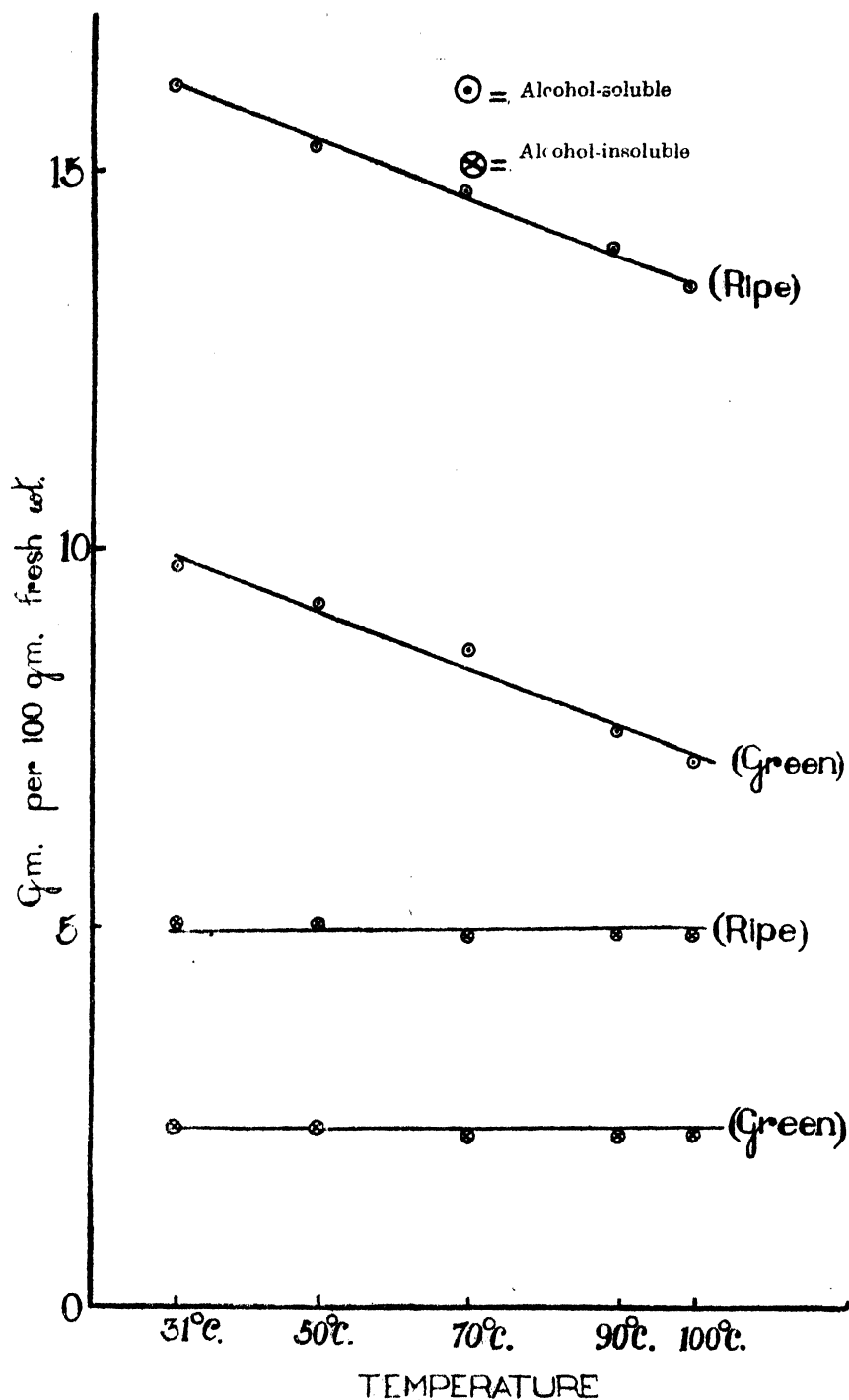


FIG. 1. Effect of temperature on dry weight

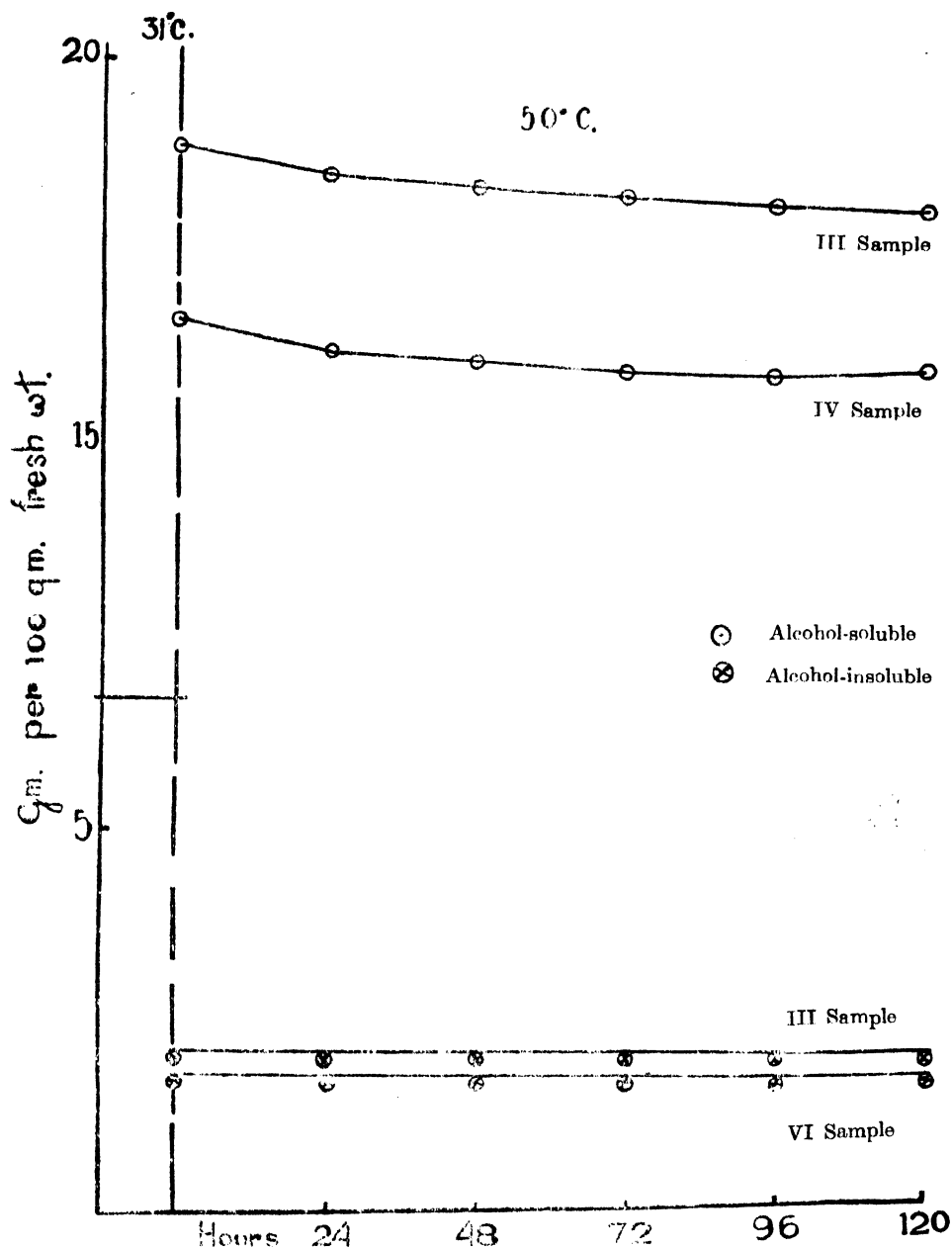


FIG. 2. Effect of time of heating on the loss of dry weight

From this observation it appears that drying at 50°C. for 24 hours at atmospheric pressure is suitable for dry weight determination of alcohol-soluble and insoluble portions of mango pulp. If temperatures higher than 50°C. are employed, there is the chance of loss of a considerable portion of alcohol soluble material in the form of a volatile compound.

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# RIPENING CHANGES IN SOME IMPORTANT VARIETIES OF GRAPES

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(With four text-figures)

**I**N any investigation of the quality of the final product which is influenced by the nature of the raw material, it is desirable to correlate physico-chemical data, as far as is possible, with the quality of the final product. In the drying of grapes and in the preparation of grape juice, for instance, it is important to determine the stage of maturity at which the grapes should be picked to give a high quality product. It was, therefore, thought desirable to make a preliminary biochemical study of the ripening changes in two important varieties of grapes, namely, the Kishmish and the Haitha.

In a study of the relation of maturity to yield and quality, in the case of Muscat grape used for drying under Californian conditions, Bioletti [1915] pointed out that grapes harvested immature yield not only a smaller total amount of dried product but also raisins of smaller size and of poorer colour and texture. According to Cruess [1938], although it is customary to begin harvesting the Muscat grape for drying in California at about 21° Balling, it is possible to delay harvesting until the grapes have attained at least 24-25° Balling. The red wine grapes to be blended with the Muscat should be gathered before full maturity in order that the juices may be of higher acidity. Petite Sirah, Alicante Bouschet, Barbera and similar varieties are generally gathered at 18 to 20° Balling. Nichols and Christie [1930], in a study of the dehydration of grapes in California, noticed that, in the case of Thompson grape, the sugar content increased with increase in Balling degree of the juice, while moisture content and acid decreased. In the case of the Muscat grape, however, the sugar content of the raisins made no consistent increase with Balling degree of the juice, although the yield, acid and the weight per raisin showed trends in the same direction as those of the Thompsons. Although their results generally confirmed the conclusions drawn by Bioletti [1915], they pointed out that the differences in yield, observed in the case grapes from two different districts, might be the result of more favourable climate, soil or crop conditions, for these varieties. Similar results have been observed by the author in a critical investigation of the drying of the Kishmish and the Haitha grapes [Siddappa, 1941].

According to Winkler [1932], except for the work of Bioletti [1925], little or no attempt appears to have been made to determine directly the correlation between the palatability—eating quality—of the analysed fruit and its chemical components such as sugars or acids. Their conclusion was that the Balling

hydrometer test is the simplest and the most reliable that can be used, particularly in determining the degree of ripeness of grapes for the purpose of standardization. According to their data, the Emperor was good when it reached 19° Balling, the Thomson seedless (Sultanina) when it reached 18° Balling and the Malaga and Tokay, when they reached 21° Balling. It will be noticed that these standards are rather low for Kishmish and Haitha grapes, which should attain at least 23 and 20° Balling respectively, to be considered fit for table purposes. The degrees Balling indicated by Bioletti and Zion for the varieties named were not, however, accepted by the growers, presumably because they were considered to be too high. Winkler's work further showed that the influence of differences in the seasonal temperatures on composition and palatability was very similar to that of regional conditions. In cool season, the acidity was relatively high in relation to Balling degree, and in hot seasons, it was relatively low, so that in hot seasons, the fruit became palatable at a lower Balling degree than in cool seasons. By combining degree Balling and per cent acidity of the expressed juice in the form of a Balling acid ratio, a highly satisfactory index of maturity was obtained. Table I gives Winkler's standards of maturity for some important table grapes.

Myers and Caldwell [1939], in a study of the preparation, by blending, of new types of unfermented grape juices other than Concord juice, give the chemical composition of forty-six important varieties of grapes obtained from the grape variety collection of the United States Department of Agriculture at Arlington Experiment Farm, Arlington, Virginia and the U. S. Horticultural Station, Beltsville, Maryland. Their data are, however, for the hot pressed juice obtained from grapes harvested when judged to be fully ripe, and do not deal with ripening changes.

#### MATERIAL AND METHODS

The samples of grapes used in the present investigation were, unless otherwise stated, mostly obtained from the vineyard at the Government Fruit Experiment Station, Quetta, although a few samples of varieties other than the Kishmish and the Haitha were obtained from vine yards situated in Gulistan, Pishin and other districts which specialize in grape growing. At the Fruit Experiment Station, the Kishmish and the Haitha grape vines, from which the weekly samples were collected during the 1939 and 1940 seasons, are grown by the cane system against wooden supports, although the local practice is to grow them in trenches. In the case of these two important varieties of grapes for which complete data are given, regarding the changes in composition during their ripening, the samples were collected at intervals of about seven days, from the very early stages of the fruit set to almost the last stage of ripening, when the berries became yellow and showed signs of drying up or deterioration.

#### *Collection of samples*

Two vines of each of the two varieties of grapes were marked off in the vineyard, and at intervals of about a week, in the early morning, one or two bunches were picked and brought immediately into the Laboratory for analysis. During the later stages of the ripening, when the grapes were approaching the palatable stage, the bunches were covered with thin muslin bags to prevent

damage by insects. It may be mentioned that no attempt was made to collect weekly random samples for analysis from a large lot of grapes, although, in a few cases, the samples were collected at random from a large consignment of the grapes. The slight fluctuation in the data presented may, therefore, be attributed to the smallness of the samples taken for analysis. Although the work was done during three seasons, to study the seasonal variations, complete analytical data for the last two seasons only are given in this paper.

TABLE I  
*A suggested standard of maturity for table grapes*  
(After A. J. Winkler)

Serial No.	Variety	Minimum degree Balling requirement	Balling acid ratios for fruit up to 20° Balling
1	Thompson's Seedless . . . . .	17	25 : 1
2	Malaga . . . . .	17	25 : 1
3	Ribier . . . . .	16	25 : 1
4	Ohanez . . . . .	17	30 : 1
5	Cornichon . . . . .	17	30 : 1
6	Muscat . . . . .	17	30 : 1
7	Emperor . . . . .	17	30 : 1
8	Tokay . . . . .	17	35 : 1
9	Olivette Blanche . . . . .	17	35 : 1
10	Molinera (Red Malaga). . . . .	16	35 : 1
11	Rish Baba . . . . .	16	40 : 1

#### *Percentage of grapes in the bunch*

The weight of the bunch was recorded and the berries carefully separated from their pedicels or caps, counted, weighed to the nearest tenth of a gram, and their weight expressed as a percentage of the total weight of the bunch. The percentage of stems and caps was obtained by difference. Any wide variation in the size of the berries, generally in the case of the Haitha grapes, was carefully recorded.

#### *Average weight of 100 grapes*

The weight of a grape is a definite index of its size and is easier to determine and far more valuable for comparative purposes than its actual linear or volume measurement. The importance of this type of determination in any

biochemical investigation of ripening changes in fruit and vegetables has been emphasised by Siddappa and Adam [1935] and Adam and Siddappa [1936], in their studies of the ripening of green peas.

The chemical composition, calculated on the basis of analysis of a known number of units, is a far more valuable aid to the visualization of the actual changes that occur during the ripening of the unit, than mere percentages. In a few cases, the standard deviation of the mean for the weight of 100 grapes is also given. The standard deviation of the mean was calculated by using the formula  $\sqrt{\frac{\sum d^2}{n(n-1)}}$ , in a series of six determinations, choosing at random 100 grapes for each weighing. The weight of the grapes was determined soon after the collection of the day's sample, in order to avoid any considerable variation in the weight, due to respiration, after detaching the bunch from the vine.

#### *Total solids*

The figures given under total solids are only comparative, since the determination was carried out on the cold expressed juice, using a Brix hydrometer. As has been previously mentioned in this paper, the Balling or Brix degree of the juice is a valuable index in following up the changes during ripening in the case of the grapes. The Brix value of the juice gives, although not exactly, the percentage of total sugars in the juice, since grape juice contains mainly sugars together with only a small percentage of nitrogenous and other substances, and mineral salts. According to Cruess [1934], however, in grape juice, the Brix or Balling value indicates the total amount of dissolved material in the juice, expressed as sugar, although about 2 to 3 per cent, in most cases, consists of things other than sugar, namely, cream of tartar, tartaric acid, protein, tannin, gums, mineral salts, etc. The Brix values given in this paper are corrected for any temperature difference at the time of their determination.

#### *Yield of juice*

While following up the ripening changes, it is highly desirable to determine the percentage of juice in the grapes. For this purpose, one hundred grams of berries were taken and placed in muslin cloth, about six inches square, previously moistened with juice from another lot of grapes under analysis, to prevent loss of yield due to absorption of the juice from the experimental lot. Water was not added to moisten the press cloth to avoid any likely dilution of the juice during pressing, as this juice was used for the determination of total solids, acidity, specific gravity, etc. The berries were crushed in the cloth, using a porcelain mortar, and the juice pressed by hand into a glass beaker. There was a certain amount of loss of juice due to spilling, adhesion, etc. during the process of extraction and its extent, as determined by weighing the pomace from the pressing, was about 6 to 8 per cent, generally. The figures given for the percentage of yield of juice are, therefore, comparative only. The exact percentage of yield will, however, be slightly higher than those given under that head. In all the analyses, the estimation of the yield of juice was carried out by the same individual, using, as far as was possible, the same standard procedure of extraction, to avoid any great variations due to personal factors, such as the amount of pressure used, number of pressings, etc.

### *Acidity*

The juice from the determination of the percentage yield was allowed to settle and the clear supernatant liquid taken for titration against standard alkali, using phenolphthalein as internal indicator. In all the acidity determinations, the settled juice was taken for titration, as the tannin in the fibre, etc., reacts with the alkali. In the case of coloured juices, where phenolphthalein could not satisfactorily be employed as internal indicator, titration was finished using it as external indicator. The figures given for the percentage acidity of the juice are as grams of tartaric acid in 100 c.c. of the juice.

### *Specific gravity of the juice*

During the 1939 analyses, the specific gravity of the juice was determined by weighing accurately 20 c.c. of the juice whose temperature at the time of estimation was recorded. The figures given are not quite exact, as a pipette, instead of a specific gravity bottle, was used to take 20 c.c. of the juice for weighing. The same pipette was, however, used in all the determinations. Weighings were completed, as rapidly as possible, to avoid any wide variation in weight due to evaporation, etc.

### *Brix-acid ratio*

The Brix-acid ratio is the ratio between the Brix reading of the juice and its percentage acidity, by volume, as tartaric acid. Although, as has been previously mentioned, the Brix value of the juice is a valuable index in the standardization of the grapes, the Brix-acid ratio appears to be a better index of maturity, as it combines the two important factors responsible for the so-called quality of the grapes. High class dessert grapes generally have a Brix-acid ratio which can be fixed fairly accurately for each variety. Grapes that are too rich in sugars or too low in acidity, or *vice versa*, are not generally considered to be suitable for table purposes.

## RIPENING CHANGES IN KISHMISH GRAPES

The results of analysis of Kishmish grapes throughout the ripening period are given in Tables III and IV. Fig. 1 shows the relation between the Brix values of the juice and the time of sampling, for the two seasons, namely 1939 and 1940, while the curve for percentage acidity is for the 1939 season only. Fig. 2 shows the Brix-acid ratio in relation to the ripening time. The break in the curves indicates the stage at which the vine, from which the samples were collected was changed.

### *Effect of season*

It will be noticed that the analytical data for the 1939 season, which was a normal year, are more regular and consistent than those for the 1940 season which was marked by abrupt spells of very warm weather during the ripening period. The effect of these sudden changes in the weather is to shift the Brix curve for 1940, slightly above the corresponding curve for 1939, thus indicating an increased photosynthetic activity and consequent accumulation of sugars during those spells of warm and bright weather. The fluctuation of the Brix curve for 1940, indicating the completion, at a very early stage, of the sigmoid curve typical of biological growth, may be attributed to these sudden

bursts of warm weather during the early stages of ripening. The meteorological data for the months of July, August and September for 1939 and 1940 are given in Table II. The effect of season on the Brix value of the juice appears, therefore, as a shift in the Brix-time curve, warmer weather during the ripening period leading to earlier maturity. In other words, the juice will attain, during a warm season, a given Brix value, earlier than in a normal season.

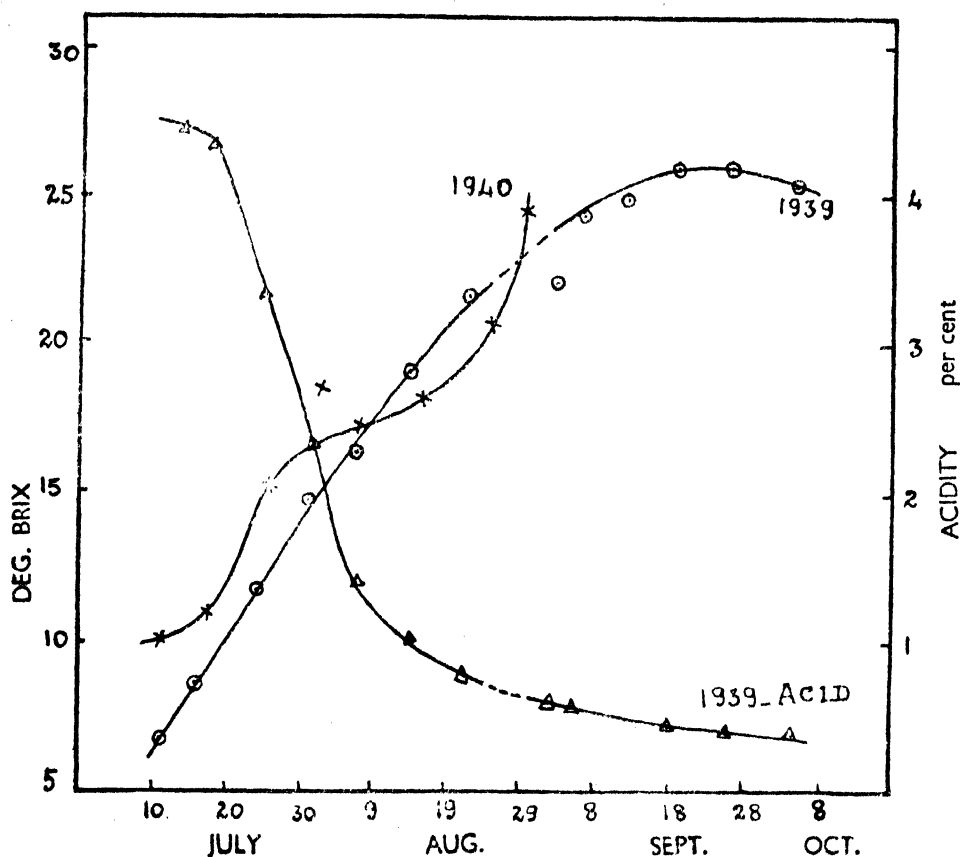


FIG. 1. Brix and acidity changes in Kishinish grapes

### *Brix value*

The Brix-time curve for 1939 is a typical S-shaped one. The Brix value of the juice increases steadily throughout the ripening period, reaching a maximum only towards the final stage, when a slight tendency to fall off may be noticed. This may be due either to a slight fall in the sugars through respiration of the grapes, the vine no longer making up for this loss, or it may be due to a certain amount of flow of the solids of the juice back into the vine. The possibility of a slight dilution of the juice, leading to a fall in the Brix value, is, however, excluded, because there is actually a fall in the weight of the berry as a result of drying up. The utilization of the sugars and other constituents of the juice to build up the non-soluble tissues of the berry, which do not appear in the Brix reading, is another possibility which cannot be ignored.

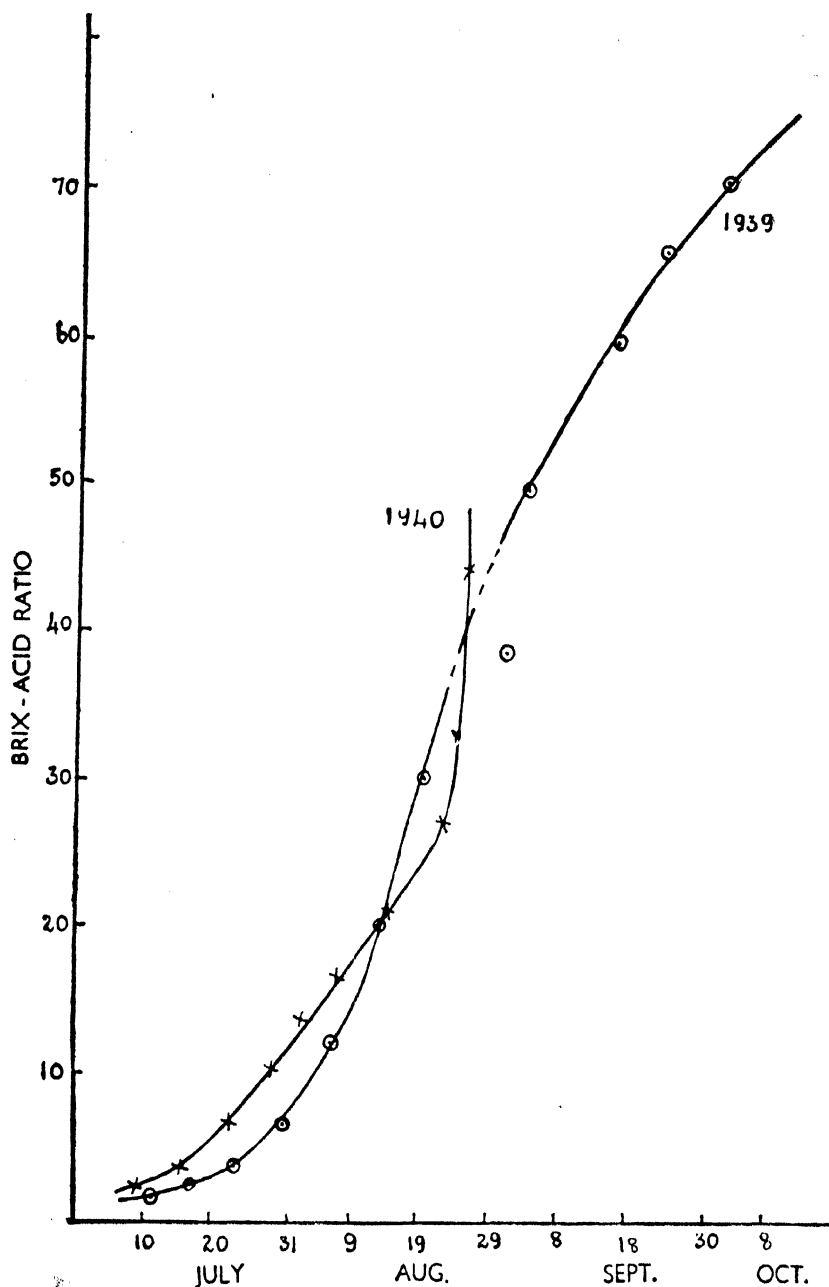


FIG. 2. Brix-acid ratio for Kishmish grapes

Kishmish grapes are considered to be 'eating-ripe' when their juice attains a value of about 23-24° Brix, although, towards the end of the season, the juice may have a Brix value as high as 26-27°. There will be a certain amount of increase in the yield, if the grapes are allowed to attain this degree of ripeness, but this increased yield will not be enough to offset the increased

prices prevailing during the earlier stages of the season. It, therefore, becomes a first rate problem to reconcile between these two opposing factors of price and yield. The keeping quality of the crop is another important consideration in any attempt to fix certain definite standards for the profitable harvesting of the grapes for table purposes. For drying, however, grapes should attain at least a Brix value of 23-24°, if the final dried product is to be of excellent quality.

TABLE II  
*Meteorological data*

Date	July 1939		July 1940		Aug. 1939		Aug. 1940		Sept. 1939		Sept. 1940	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1	91	66	95	59	93	56	92	66	93	55	86	47
2	91	66	96	63	92	54	91	60	94	50	86	47
3	86	62	98	65	94	59	93	66	92	48	88	49
4	89	65	97	61	94	57	91	65	92	50	89	48
5	94	64	95	56	95	63	90	64	94	53	89	47
6	94	62	95	55	95	66	91	58	93	52	89	49
7	96	61	95	56	95	59	94	66	94	60	89	49
8	95	64	95	63	88	62	95	67	92	59	89	45
9	90	56	95	68	88	63	92	60	92	57	88	51
10	92	70	95	65	90	55	84	56	90	55	86	51
11	95	71	96	71	91	52	88	54	88	57	85	58
12	96	62	96	66	94	57	84	54	84	47	78	51
13	97	60	95	68	96	63	90	59	80	44	82	46
14	98	70	96	69	95	64	88	54	79	42	84	57
15	95	70	96	68	95	65	91	67	84	45	84	57
16	90	61	94	68	95	63	91	68	86	45	85	47
17	90	61	94	69	92	59	83	69	87	48	86	48
18	90	63	95	63	91	55	88	69	86	51	88	47
19	93	59	95	71	89	55	87	55	85	48	88	50
20	95	62	94	72	89	50	87	54	86	45	89	47
21	94	61	91	70	91	53	89	56	87	49	88	47
22	94	59	95	65	94	55	89	59	87	49	89	47
23	95	62	95	62	94	59	88	53	85	47	86	47
24	96	60	90	72	95	61	87	55	83	46	86	50
25	96	59	92	69	95	69	88	56	83	41	85	56
26	97	63	92	69	95	68	90	54	83	41	82	52
27	96	69	95	69	95	67	89	50	87	39	79	47
28	92	68	92	66	96	67	88	50	83	41	81	54
29	91	58	95	65	95	64	86	51	84	40	78	52
30	91	62	96	67	95	64	89	46	83	43	77	50
31	92	61	93	63	94	57	88	47	...	...	...	...

TABLE III  
*Ripening changes in Kishmish grapes (1939)*

Experiment No.	Date	Weight of bunch (gm.)	Percent- age of grapes in the bunch	Average weight of 100 grapes (gm.)	Yield of juice (per cent)	Juice		Brix- acid ratio	Remarks
						Total solids ("Brix)	Acidity as tartaric (per cent)		
1	12 July	147	89.8	41.8 $\pm$ 1.22	70.0	6.7	4.40	1.5:1	*Juice pressed by the laboratory attendant, hence the variation
2	17 "	118	92.4	58.8 $\pm$ 0.48	57.0*	8.6	4.29	2.0:1	
3	24 "	198	96.0	68.5 $\pm$ 0.84	74.0	12.0	3.31	3.6:1	
4	31 "	218	94.0	78.3 $\pm$ 0.50	59.0*	15.1	2.32	6.5:1	
5	7 Aug.	302	94.4	106.0 $\pm$ 0.50	74.7	16.5	1.40	11.8:1	Still unripe
6	14 "	205	95.6	116.0 $\pm$ 1.10	74.7	19.2	0.97	19.8:1	
7	21 "	192	94.8	130	72.7	22.0	0.74	29.7:1	Greenish yellow berries, nearing eating ripe stage A different vine grown in a trench was chosen, as all the bunches had been removed from the first vine by mistake by the pickers
8	2 Sept.	232	95.7	123	71.0	22.0	0.57	38.6:1	
9	5 "	237	95.8	111	69.5	24.7	0.50	49.4:1	
10	18 "	250	..	120	70.0	26.2	0.44	59.5:1	
11	25 "	315	..	125	75.0	26.5	0.40	66.2:1	Fully ripe
12	6 Oct.	..	..	124	..	28.7	0.57	50.4:1	Kandahar grape; fully ripe and yellow

TABLE IV  
*Ripening changes in Kishmish grapes (1940)*

Experiment No.	Date	Weight of bunch (gm.)	Percentage of grapes in the bunch	Average weight of 100 grapes (gm.)	Yield of juice (per cent)	Yield of pomace (per cent)	Juice		Brix-acid ratio	Remarks
							Total solids ("Brix)	Acidity as tartaric (per cent)		
1	11 July	110	91.8	60.0	76.0	21.0	10.0	4.07	2.5:1	Not eating ripe yet. Warm weather
2	18 "	146	93.8	53.0	66.0	30.0	10.5	4.24	2.5:1	
3	25 "	170	93.0	73.0	75.0	16.0	15.1	2.52	6.0:1	
4	2 Aug.	144	93.7	98.0	74.0	14.0	18.5	1.38	13.4:1	
5	8 "	154	95.5	79.0	79.0	13.0	17.5	1.07	16.4:1	Warm weather during the past week. Kishmish grapes from Kandahar are available these days
6	15 "	139	95.0	89.0	84.0	10.0	18.0	0.86	20.9:1	
7	24 "	149	94.0	82.0	78.0	15.0	20.5	0.77	26.6:1	
8	28 "	180	96.7	114.0	78.0	12.0	24.5	0.56	43.8:1	

Ditto

### *Acidity*

The percentage of acidity falls regularly throughout the ripening period, the fall being rather steep during the early stages and almost flat towards the end. 'Eating ripe' Kishmish grapes have an acidity of about 0.4 to 0.5 per cent, although in the early stages, when the berries are small, it may be as high as about 4.5 per cent. There is only a very slight fall in the acidity after the eating-ripe stage of ripeness has been reached.

### *The Brix-acid ratio*

The Brix-acid ratio is a highly characteristic index of the quality of the grapes. It will be seen, from Tables III and IV. and Fig. 2, that it increases steadily throughout the ripening period, changing, during the 1939 season, from 1.5:1 to nearly 70:1. The variation during the 1940 season was, however, from 2.5:1 to 43.8:1 only, the ripening changes having not been followed completely, as in the previous season. The curve for 1940 is less regular than that for 1939 due to sudden changes in the weather during ripening. When the grapes are at about the eating ripe stage, the ratio is in the neighbourhood of nearly 40:1. Grapes having a ratio far below this are not quite fit for table purposes, and those that have a ratio far above are generally over-ripe, and, although fit for eating, do not have long storage life. Tentatively, it may be stated, at this stage of our experience, that Kishmish grapes of good dessert quality should have a Brix-acid ratio of about 40:1. It may be noted that this ratio for dessert quality Kishmish grapes is higher than that for most Californian grapes (Table I), probably because of their very high sugar content and correspondingly low acidity. Kishmish grapes from Kandahar that are usually sold at Quetta during the peak of the grape season have a Brix-acid ratio of about 50:1. They are generally richer both in sugars and in acidity than the ones from Quetta vineyards. The higher Brix reading of the Kandahar grape may be partly due to a slight drying up of the berries during transit and storage, or it may be a function of factors due to variations in soil, climate or cultural practice.

### *Percentage of berries in the bunch*

The percentage of grapes in the bunch increases as the ripening advances, but the change is small. Eating ripe bunches of Kishmish grapes contain about 95 per cent, by weight, of berries, the stems and caps forming about 5 per cent of the total weight of the bunch.

### *Yield of juice*

The yield of juice, as given in the tables, is what is obtained in small laboratory experiments and is comparative only. Under large scale trials, where the bunches are crushed in a grape crusher and pressed in basket presses, the yield of juice from ripe grapes may not exceed about 60—65 per cent, even after two or three pressings. With hydraulic pressure, it may, however, reach as high as 70—75 per cent by weight of the fresh grapes.

## DEVELOPMENT OF A SINGLE KISHMISH BERRY

It is highly interesting and instructive to trace the development of the various constituents in a single berry throughout the ripening period. The

results of analyses for the seasons 1939 and 1940 are given in Tables V and VI. The total soluble solids in 100 grapes are calculated from the average weight of the berries, the percentage yield of juice and the Brix reading and are, therefore, approximate only. The figures for the amount of acid are deduced in a similar manner. In Table V, however, the acidity has been corrected for the specific gravity of the juice, since the percentage of acidity as given in Table III is on a volume basis only.

It will be noted that the average weight of the berry increases rapidly in the early stages of development and gradually towards the end. The total soluble solids increase in a similar manner, while the acidity decreases continuously, although during 1939, a slight increase is noticed in the early stages. During the very early stages of ripening, the acids accumulate to a certain extent, but towards the last stages, they are readily used up in the synthesis of other constituents.

The total soluble solids-acid ratio is the same as the Brix-acid ratio, discussed previously, although it is slightly higher, when the figures for the absolute acidity of the berry are corrected for the specific gravity of the juice. The difference between the two ratios increases with the increase in the specific gravity of the juice and hence with advancing state of maturity. In the standardization of grapes, the Brix-acid ratio is, however, more direct and more easily deduced than the total soluble solids-acid ratio.

TABLE V  
*Development of Kishmish grapes (1939)*

Experiment No.	Date	Average weight of 100 grapes (gm.)	Total soluble solids in 100 grapes (gm.)	Acid in 100 grapes as tartaric, corrected for sp. gr. (gm.)	Total soluble solids-acid ratio
1	12 July . .	41.8	1.96	1.26	1.56
2	17 „ . .	58.8	2.88	1.41	2.04
3	24 „ . .	68.5	6.08	1.61	3.78
4	31 „ . .	78.3	6.97	1.02	6.83
5	7 Aug. . .	106.0	13.06	1.05	12.44
6	14 „ . .	116.0	16.63	0.79	21.05
7	21 „ . .	130.0	20.79	0.65	32.06
8	2 Sept. . .	123.0	19.22	0.46	41.78
9	5 „ . .	111.3	19.10	0.35	54.56
10	18 „ . .	120.0	22.01	0.33	66.70
11	25 „ . .	125.0	24.84	0.34	73.07

TABLE VI  
*Development of Kishmish grapes (1940)*

Experi- ment No.	Date	Average weight of 100 grapes (gm.)	Total soluble solids in 100 grapes (gm.)	Acid in 100 grapes, as tartaric, (not corrected) (gm.)	Total soluble solids-acid ratio
1	11 July . .	60.0	4.56	1.86	2.45
2	18 „ . .	53.0	3.67	1.48	2.48
3	25 „ . .	73.0	8.27	1.38	5.99
4	2 Aug. . .	98.0	13.42	1.00	13.42
5	8 „ . .	79.0	10.91	0.67	16.29
6	15 „ . .	89.0	13.46	0.64	21.03
7	24 „ . .	82.0	13.11	0.49	26.75
8	28 „ . .	114.0	21.79	0.50	43.58

#### RIPENING CHANGES IN HAITHA GRAPES

The results of analyses of Haitha grapes, during the 1939 and 1940 seasons, are given in Tables VII and VIII. It will be noticed that the data are more or less similar to those recorded in the case of Kishmish grapes, although a few minor changes can be seen in some of the items. Although the bunches are larger in size than those of the Kishmish, the percentage of berries is not far different. The average weight of a single ripe berry may be as high as 5 grams. The yield of juice varies from 61.0 to 77.0 per cent throughout the period of ripening. The Brix reading of the juice increases and the percentage acidity decreases, throughout the period of ripening, the curves (Fig. 3) being typically S-like. The Brix readings for 1940 are higher than the corresponding ones for 1939, as in the case of the Kishmish grapes, the differences being due to seasonal factors, as has been explained previously. The slight scatter noticed in the plotted readings both for Brix reading and the percentage acidity may be due to the unavoidable variation in the experimental material itself, the bunches of grapes often containing a large percentage of berries far below the average size. The effect of this natural variation in size in the experimental material was, however, avoided, as much as possible, by rejecting almost all those berries that differed widely from those of average size in the bunch. The Brix-acid ratio (Fig. 4) rises slowly in the early stages of development and more rapidly as the eating ripe stage is being reached, after which the increase is rather less rapid. Under local conditions, Haitha grapes having a Brix reading of about 19° and 0.46-0.47 per cent acidity are considered fit

for eating, although samples of these grapes, from Gulistan and other places, have been found to have a juice of about  $23.5^{\circ}$  Brix and  $0.28$  per cent acidity. The low Brix reading attained by Haitha grapes may be due to the effect of soil or climate, or it may be due to cultural practice, as has been pointed out elsewhere [Siddappa, 1941]. The Brix-acid ratio of eating ripe Haitha grapes will be about  $40 : 1$ , although it may be as high as  $80 : 1$  in certain cases. This ratio is about the same as for Kishmish grapes, as the Haitha grape is low both in its Brix and acidity. During the 1939 analyses, the Brix reading of the juice even on 19 September was only  $19^{\circ}$ . The grape season ends by about the end of September and even if the berries are allowed to ripen still further, the Brix value may increase slightly, but the season will become unduly short and uneconomical.

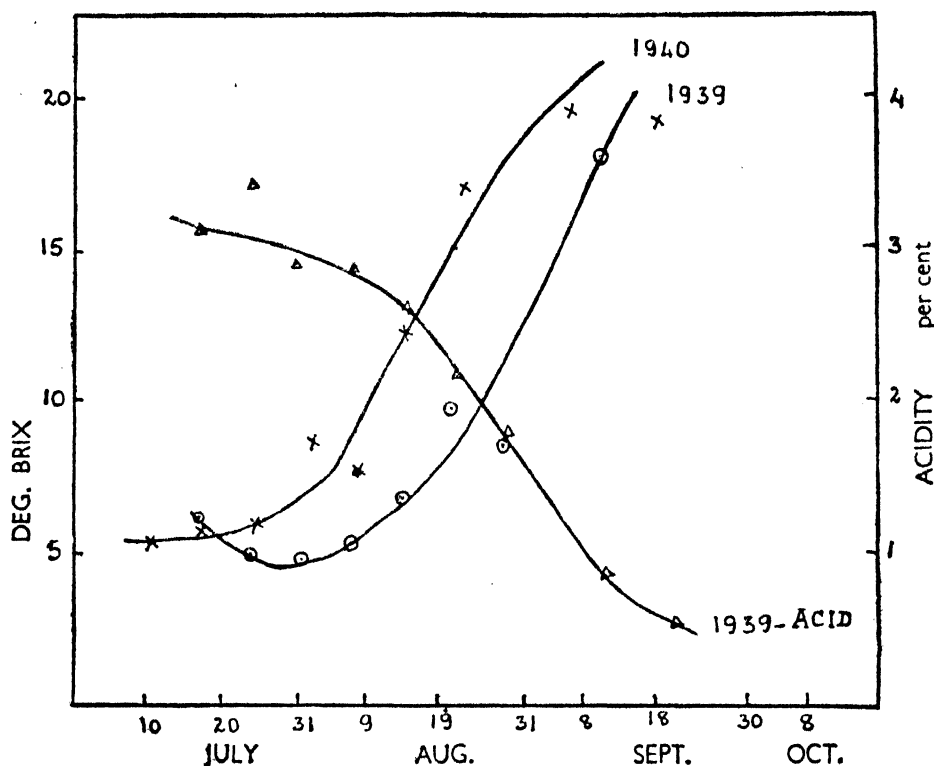


FIG. 3. Brix and acidity changes in Haitha grapes

#### DEVELOPMENT OF A SINGLE HAITHA BERRY

Changes in total soluble solids, absolute acidity, etc. during the development of a single Haitha berry are given in Tables IX and X. As in the case of the Kishmish grape, the data are calculated on the basis of development of 100 berries. It will be noticed that the results are similar to those recorded in the case of the Kishmish grape, although the figures for total soluble solids

and acidity are comparatively higher due to the naturally bigger size of the Haitha grape. The total soluble solids increase and the absolute acidity decreases, throughout the period of ripening under observation. As in the case of the Kishmish grape, there is a slight tendency for the acids to accumulate during the very early stages of development of the berry. The total soluble solids-acid ratio is the same as the corresponding Brix-acid ratio, the percentage acidity figures having not been corrected for the specific gravity of the juice.

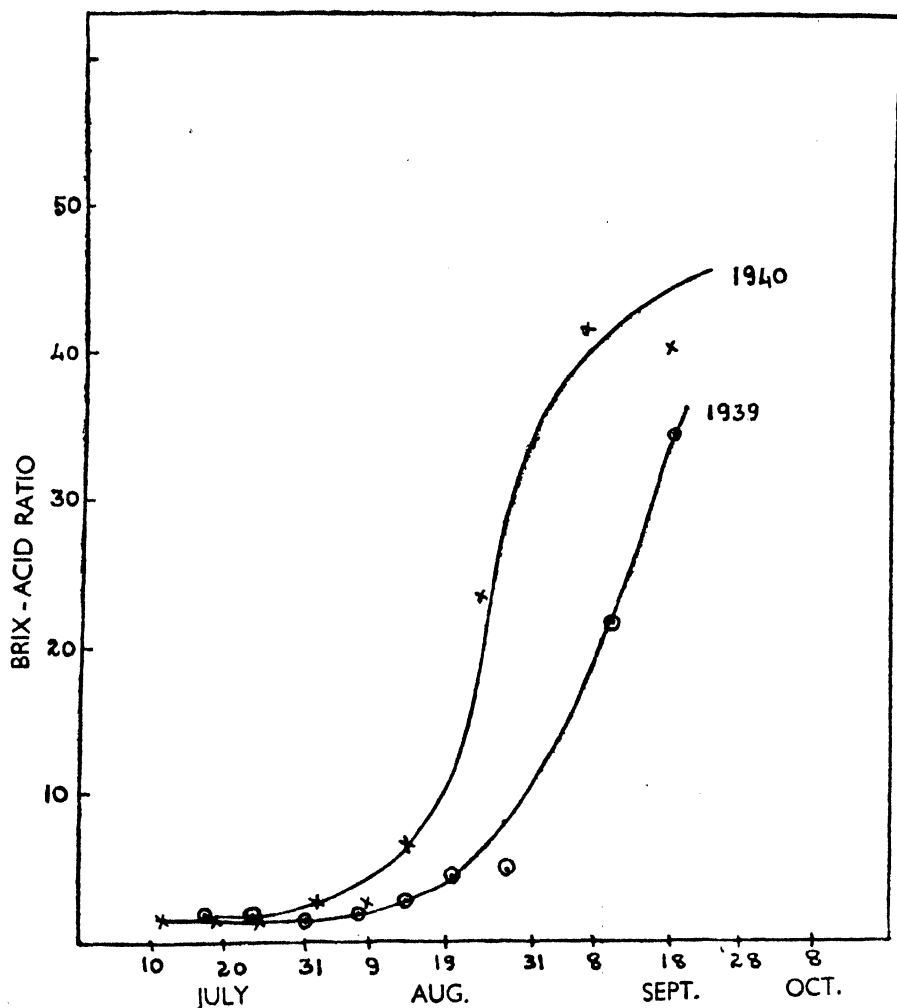


FIG. 4. Brix-acid ratio for Haitha grapes

TABLE VII

*Ripening changes in Hailha grapes (1939)*

Experi- ment No.	Date	Weight of bunch (gm.)	Percent- age of grapes in the bunch	Average weight of 100 grapes (gm.)	Juice			Brix- acid ratio	Remarks
					Yield (per cent)	Total solids (°Brix)	Acidity as tartaric (per cent)		
1	17 July	257	95.0	169.3 + 4.6	61.0	6.0	3.12	1.9:1	
2	24 "	400	94.8	172.3 + 3.7	70.3	4.7	3.41	1.4:1	
3	31 "	385	92.9	176.0 + 3.8	66.7	4.5	2.92	1.5:1	
4	7 Aug.	468	94.0	186.0 + 3.4	71.3	5.0	2.85	1.8:1	
5	14 "	410	95.6	231.7 + 1.3	74.7	6.5	2.48	2.6:1	
6	21 "	342	87.1	251	74.8	9.2	2.11	4.4:1	
7	28 "	307	92.8	252	72.8	8.0	1.68	4.8:1	
8	11 Sept.	277	95.3	470	71.0	17.7	0.82	21.6:1	Sudden change in Brix-acid ratio
9	18 "	688	..	542	77.0	17.7	0.51	34.7:1	Not yet fit for dessert purposes
10	25 "	..	..	..	..	..	..	..	Unfortunately, somebody had removed all the bunches from the experimental vines

TABLE VIII  
*Ripening changes in Haiha grapes (1940)*

Experiment No.	Date	Weight of bunch (gm.)	Percent- age of grapes in the bunch	Average weight of 100 grapes (gm.)	Yield of juice (per cent)	Yield of pomace (per cent)	Juice			Remarks
							Total solids (°Brix)	Acidity as tartaric (per cent)	Brix- acid ratio	
1	11 July	215	96.3	147	67.0	23.0	5.2	3.34	1.6:1	Juice from whole lot, including small berries
2	18 "	305	92.5	187	74.0	20.0	5.8	3.53	1.6:1	
3	25 "	263	95.8	172	68.0	20.0	5.7	3.43	1.7:1	Very warm weather. Large number of berries below average size
4	2 Aug.	160	95.6	200	74.0	18.0	8.4	3.04	2.8:1	Warm weather during the past week. Small bunch
5	9 "	{ 115+ 145	93.5	169	73.0	19.0	7.5	3.00	2.5:1	Two bunches were taken
6	15 "	172	95.4	248	78.0	15.0	12.0	1.75	6.9:1	Not yet eating ripe
7	24 "	550	94.9	345	84.0	15.0	16.7	0.71	23.5:1	Numerous small berries in the bunch
8	7 Sept.	525	97.1	510	80.0	12.0	19.2	0.46	41.8:1	Eating ripe stage; greenish yellow berries
9	19 "	680	..	496	..	..	19.0	0.47	40.4:1	Eating ripe stage
10	26 "	..	..	..	..	..	..	..	..	All the bunches had been removed by somebody

TABLE IX  
*Development of Haitha grapes (1939)*

Experi- ment No.	Date	Average weight of 100 grapes (gm.)	Total soluble solids in 100 grapes (gm.)	Acid in 100 grapes, as tartaric (gm.)	Total soluble solids-acid ratio
1	17 July . .	169.3	6.20	3.22	1.92
2	24 „ . .	172.3	5.69	4.13	1.38
3	31 „ . .	176.0	5.28	3.43	1.54
4	7 Aug. . .	186.0	6.63	3.78	1.76
5	14 „ . .	231.7	11.25	4.29	2.62
6	21 „ . .	251.0	17.28	3.96	4.36
7	28 „ . .	252.0	14.68	3.08	4.76
8	11 Sept. . .	470.0	59.07	2.73	21.60
9	18 „ . .	542.0	73.87	2.13	34.70

TABLE X  
*Development of Haitha grapes (1940)*

Experi- ment No.	Date	Average weight of 100 grapes (gm.)	Total soluble solids in 100 grapes (gm.)	Acid in 100 grapes, as tartaric (gm.)	Total soluble solids-acid- ratio
1	11 July . .	147.0	5.12	3.29	1.56
2	18 „ . .	187.0	8.12	4.88	1.64
3	25 „ . .	172.0	6.67	4.01	1.66
4	2 Aug. . .	200.0	12.43	4.50	2.76
5	9 „ . .	169.0	9.25	3.70	2.50
6	15 „ . .	248.0	23.21	3.39	6.86
7	24 „ . .	345.0	48.40	2.06	23.52
8	7 Sept. . .	510.0	78.34	1.88	41.74
9	19 „ . .	496.0	..	..	..

## ANALYSIS OF SOME IMPORTANT LOCAL VARIETIES OF GRAPES

Although the Kishmish and the Haitha are the two most important grapes grown in Baluchistan, there are a number of other varieties grown on a fairly large scale, in some of the grape-growing tracts of the province. They are generally seeded white grapes, although a few like Tore, Sahebi and Khair-e-Ghulama are black or purplish black grapes. It was, therefore, thought desirable to collect a number of samples of these varieties, especially at the time of harvesting, and find out their Brix-acid ratio, in order to be able to draw up some empirical standards of maturity for the profitable harvesting of at least a few of the important varieties of grapes grown in the Province. The results are given in Table XI. It will be noticed that fully ripe white grapes are generally high in sugars and low in acidity, thus giving a comparatively high Brix-acid ratio, while the black grapes from Quetta and Pishin are high both in sugars and in acidity, giving a comparatively low Brix-acid ratio. The Sahebi which is a pale purple coloured grape, with seeded oval berries, has a characteristic flavour and is, therefore, highly valued as a table grape. When eating ripe, its juice has a Brix reading of about 20° and an acidity of about 0.5 per cent. Dark purple or black grapes are generally known locally as Tore and their nomenclature is, therefore, not definite. The highest Brix reading recorded in the table, namely, 28.7° was for a sample of Kishmish grapes from Kandahar, and even at this high value, the acidity was as high as 0.57 per cent, giving a Brix-acid ratio of only 50.4 : 1. The superiority of the Kandahar Kishmish grapes, on which popular opinion places a high value, may be partly due to its peculiarly high Brix and acidity values. There is a wide variation in the Brix-acid ratio, namely from about 11 : 1 to 84 : 1, although a ratio of about 40 or 50 to one would be sufficiently accurate to define the eating-ripe stage of maturity of many of the varieties of grapes for which the data has been collected. The difficulty of fixing, at the present state of our knowledge, more definite standards will, however, be appreciated when one realizes the possibility of the wide variation in the ratio, even at the so-called eating-ripe stage.

## ANALYSIS OF SOME IMPORTANT FOREIGN VARIETIES OF GRAPES

At the Fruit Experiment Station, Quetta, a number of important varieties of grapes have been lately introduced and their performance, under local conditions, is under investigation. Samples of grapes from five of these imported varieties were analysed during the seasons of 1939 and 1940 and the results are given in Table XII. It may be pointed out that the berries were not considered to be fully ripe at the time of analysis, during both the seasons. Of the five varieties named, Emperor, Black Hamburg and Gros Colman are purple coloured seeded grapes, while Olivette Blanche is a seeded white grape and Thompson's Seedless, a small, round, seedless, white grape resembling the Kishmish in several respects. Emperor and Olivette Blanche, both from California, are reputed to be late varieties, eminently suited for cold storage and transport. Thompson's seedless is the most important white grape extensively used for drying in California and elsewhere.

It may be assumed, for the present, that many of these foreign varieties might attain, under local conditions, a Brix value of 19—20° and a Brix-acid

ratio of about 40 : 1, although in the case of Thompson's Seedless, the ratio recorded is only 15·3 : 1, due to the high acidity of the juice. At the time of analysis, the berries of this variety, although ripe and greenish yellow in colour, were very small, the average weight of a berry being 0·48 gm. only, that is, much less than about half the weight of an average Kishmish berry. It is doubtful if this grape could compete successfully with the popular Kishmish grape, unless it be for its well balanced sugar-acid ratio.

TABLE XI

*Analysis of some important local varieties of grapes (1939-1940)*

Serial No.	Date	Variety, etc.	Weight of 100 grapes (gm.)	Juice			Remarks
				(°Brix)	Acidity as tartaric (per cent)	Brix-acid ratio	
1	4-10-39	Kishmish (Quetta)	...	26·2	0·37	70·8 : 1	Ripe; seedless white grape
2	6-10-39	Kishmish (Kandahar)	...	28·7	0·57	50·4 : 1	Ripe
3	6-10-39	Do	...	26·1	0·43	60·7 : 1	Ripe
4	19-8-40	Kishmish (Quetta)	92	26·0	0·45	57·8 : 1	Yellow berries
5	19-8-40	Do	...	25·5	0·67	38·1 : 1	Slightly greenish berries
6	10-9-40	Do	...	24·0	0·48	50·0 : 1	Sample from Fruit Experiment Station
7	5-10-40	Do	...	26·8	0·55	48·7 : 1	Over-ripe grapes
8	6-8-40	Kishmish (Kandahar)	174	24·7	0·45	54·9 : 1	Early season sample
9	4-10-39	Haitha (Pishin)	...	23·0	0·28	82·1 : 1	Large white grape with seeds; ripe
10	23-10-39	Do	...	19·5	0·36	54·2 : 1	Ditto
11	28-9-39	Haitha (Gulistan)	550	23·5	0·28	83·9 : 1	Ditto
12	4-9-40	Haitha (Quetta)	...	17·3	0·52	33·3 : 1	Slightly unripe
13	10-9-40	Do	528	19·5	0·46	42·4 : 1	Ripe
14	8-9-39	Tand (Gulistan)	520	20·5	0·62	33·3 : 1	White grape; fully ripe
15	9-9-39	Do	...	22·0	0·67	32·8 : 1	Ditto
16	8-9-39	Kalmak (Gulistan)	370	23·7	0·45	52·7 : 1	Ditto
17	9-9-39	Do	...	22·6	0·52	43·5 : 1	Ditto
18	26-10-39	Khair-e-Ghulama	584	26·2	0·37	70·8 : 1	Dark purple grape; ripe
19	22-8-39	Black Monucca	302	22·0	1·27	17·3 : 1	Dark purplish grape, rich in acid; ripe
20	9-8-40	Shendukhanf	172	24·8	0·68	36·5 : 1	Long seedless Kishmish grape; ripe
21	9-8-40	Sahebi	324	16·7	0·61	27·4 : 1	Large, slightly oval berries with light purplish skin and marked flavour; not fully ripe
22	31-8-40	Do	...	19·7	0·53	37·2 : 1	Ripe
23	19-8-40	Kadak (Quetta)	320	23·1	0·76	30·4 : 1	Large round white berries; seeds present; thin skin, juicy flesh and sweet taste

TABLE XI—*contd*

Serial No.	Date	Variety, etc.	Weight of 100 grapes (gm.)	Juice			Remarks
				(°Brix)	Acidity as tartaric (per cent)	Brix-acid ratio	
24	19-8-40	Sheikh Ali	184	13.7	1.23	11.1:1	Slightly oval berries of medium size; white, seeded grape with strongly acid taste; not fully ripe
25	24-8-40	Do	360	21.0	0.70	30.0:1	Ripe berries
26	24-8-40	Hussaini	461	18.1	0.31	58.4:1	Large greenish yellow berries resembling the Haitha berries, but differing from them in being uniformly thick, with only a slight concave on one side; very sweet seeded grape; ripe
27	9-8-40	Tore	241	27.8	0.42	66.2:1	Large slightly oval berries; thick purplish black skin; seeded sweet black grape; ripe
28	24-8-40	Black grape (Gulistan).	580	22.2	0.70	31.7:1	Large round purple black berries; thick skin; seeds present; acid sweet taste; appears to be different from Tore; ripe
29	24-8-40	Black grape (Quetta)	560	20.6	0.62	33.2:1	Compact bunch; large round seeded black grape; ripe

### SUMMARY

A detailed investigation has been carried out into the changes that take place during the ripening of the Kishmish and the Haitha grapes, two of the most important varieties of grapes grown in Baluchistan. The results of analysis of a few other important varieties of grapes, local as well as foreign, are given and the limitations for fixing up definite and exact standards of maturity have been pointed out. The main results are briefly as follows:—

#### *Kishmish grapes*

In the case of Kishmish grapes, the Brix value increases steadily throughout the main period of ripening, although a slight fall may be noticed towards the very final stage, when the berry has started to dry up. The percentage of acidity falls regularly throughout the ripening period, the fall being rather steep towards the early stages and almost flat towards the end. Eating ripe Kishmish grapes have generally a Brix value of 23—24° and an acidity of about 0.4—0.5 per cent. Grapes having a considerably higher Brix reading and lower acid value are considered to be over-ripe and are of poor palatability on account of their ill balanced Brix-acid ratio.

TABLE XII

*Analysis of some important foreign varieties of grapes (1939-40)*

Serial No.	Date	Variety, etc.	Weight of 100 grapes (gm.)	Juice			Remarks
				(°Brix)	Acidity as tartaric (per cent)	Brix-acid ratio	
1	7-10-39	Black Hamburg	374	21.5	0.46	46.7 : 1	Large loose bunches; large, round, light purplish black berries; seeds present; thick skin; not fully ripe at the time of analysis
	27-10-39	Do	...	19.5	0.70	27.9 : 1	Ditto
	2-10-40	Do	...	18.0	0.37	48.7 : 1	Ditto
2	7-10-39	Emperor	354	15.7	0.50	31.4 : 1	Light purplish berries, slightly oval in shape; seeds present; berries attached by means of thick caps; late variety of grape reputed to keep long in cold storage; not fully ripe under local conditions
	14-10-39	Do	...	16.8	0.40	42.0 : 1	Ditto
	27-10-39	Do	...	17.2	0.52	33.1 : 1	Ditto
	2-10-40	Do	...	19.0	0.68	28.0 : 1	Juice extracted by the 'hot' method; hence a slight increase in Brix reading
3	7-10-39	Olivette Blanche	569	18.0	0.49	36.7 : 1	Loose bunches of medium size; long oval berries; thick skin; seeded white grape; flesh slightly astringent in taste; late variety of white grape; not fully ripe at the time of experiment, due to local conditions
	27-10-39	Do	...	16.5	0.56	29.5 : 1	
	2-10-40	Do	...	14.2	0.54	26.3 : 1	
4	7-10-39	Gros Colman	480	19.4	0.31	62.6 : 1	Purplish black grape; seeds present; not fully ripe at the time of analysis
	27-10-39	Do	...	17.4	0.37	47.0 : 1	
5	11-9-40	Thompson's Seedless	48	19.3	1.26	15.3 : 1	Small loose bunches; small, round, seedless, white berries; flesh tasting acid sweet; berries greenish yellow in colour; this is a famous foreign grape used extensively for drying; ripe berries rather very small in size

The Brix-acid ratio is highly characteristic of the grape, and when used in conjunction with the Brix reading, serves to fix certain practical standards for the maturity of the grape. Tentatively, a ratio of about 40 : 1 and a minimum Brix reading of about 23° may be used to define Kishmish grapes of good dessert quality, although grapes having a higher Brix reading and a higher ratio are not considered to be unfit for table purposes.

The percentage of berries in the bunch increases only slightly as the ripening advances and the eating-ripe bunches contain about 95 per cent by weight of berries, the stems and caps forming about 5 per cent of the total weight of the bunch.

Under experimental conditions, the percentage of juice in the berries remains practically constant throughout the period of ripening and constitutes about 80 per cent of their total weight, the remaining 20 per cent being made up of the skin and flesh.

During a comparatively warmer ripening season, the Brix values are generally higher than the corresponding ones during a normal season, the increase being mainly due to the accelerated photosynthetic activity during spells of bright and warm weather. The changes in the various constituents of the grapes during an abnormally warm season are less regular than in a normal season.

In the development of a single berry, its average weight increases rapidly in the early stages and more gradually towards the end. The total soluble solids increase in a similar manner, while the acidity decreases continuously, although there is a marked tendency for the acids to accumulate during the very early stages.

Kishmish grapes from Kandahar are generally richer than Quetta ones, both in their Brix and acidity values. This may be due to the soil or climate or changes during storage and transport.

Cultural treatments such as staking, trenching, etc. may considerably affect the changes during the development of the grapes.

### *Haitha grapes*

The changes observed during the ripening of the Haitha grapes are almost similar to those that occur in the case of the Kishmish grapes, except for a slight scatter in the data for the various constituents, due to the inherent non-uniformity of the experimental material, bunches of grapes often containing numerous berries far above or below the average size. The Brix reading increases and the percentage acidity decreases throughout the main period of ripening, the Brix readings being slightly higher in an abnormally warmer season than in a normal one. Under local conditions, eating ripe Haitha grapes have a Brix-acid ratio of about 40 : 1 and a Brix reading of 18—20° only. Haitha grapes from other parts have been found to be considerably higher in their Brix reading, as a result of climatic conditions, cultural practices, etc.

As in the case of the Kishmish berry, during the development of a single Haitha berry, the total soluble solids increase and the absolute acidity decreases throughout the ripening period and there is a marked tendency for the acids to accumulate during the early stages.

### *Other varieties*

Other local white grapes like Kadak, Hussaini, Tand, Kalmak, Sheikh Ali, etc. have, when ripe, a Brix-acid ratio widely differing from about 40 : 1, and are generally rich in sugars and low in acidity. Purple-coloured grapes like Tore and Khair-e-Ghulama are generally rich both in their Brix and acidity values and have a comparatively low Brix-acid ratio. Eating-ripe Sahebi-

grape has a Brix acid ratio of about 40 : 1 and, being highly flavoured, it is considered to be a high class table grape.

Among the foreign varieties of grapes that have been introduced into the Province, Emperor, Gros Colman and Hamburg are purple-coloured grapes, while Olivette Blanche and Thompson's Seedless are white grapes. Under local conditions, they may attain a Brix value of 19—20° and a Brix-acid ratio of about 40 : 1. At Quetta, the Thompson's Seedless is a very small grape with high acidity and low Brix-acid ratio and does not compare favourably with the Kishmish grape.

#### ACKNOWLEDGMENTS

I have great pleasure in acknowledging my indebtedness to the Imperial Council of Agricultural Research for financing this work under a general scheme of research for Fruit Canning and Preserving in Baluchistan. My grateful thanks are due to A. M. Mustafa, Esquire, Agricultural Officer in Baluchistan, for his keen interest in the course of the above investigation.

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# RESEARCH NOTE

## PRODUCTION OF FLOWERS ON ROOTSTOCK STEMS OF MANGO GRAFTS IN THE NURSERY

BY

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(Received for publication on 16 July 1941)

(With Plate XVIII)

IT appeared to be of much interest, at this station, to note during the 1941 mango season some cases of flowering from the stem of the seedling rootstock of one-year old mango grafts (inarcheds) which were yet in pots in the nursery. It is not uncommon to see the scion shoot flowering in the very first season after grafting. They usually are shoots taken from mature trees, and quite often they have fruit-buds at the time of their separation from parent trees. But, flowering from rootstock stem is rather unusual. A mango seedling does not, as a rule, flower before it is at least five to six years old.

The grafts were prepared from mature scion parents of two Bombai; three Langra and one Fazli mango trees. The seedlings used as rootstocks were of mixed origin. They were sown during the rains (June and July) of 1938 and potted in June, 1940. Inarching was done in July the same year. At the time of inarching the seedlings were 0.75–1.0 cm. in diameter at base, and 45–50 cm. in height. The grafts were finally separated from the parent trees in October. They were then kept under partial shade in the nursery.

In the spring of 1941, a good many of these grafts flowered from their scion shoots. The extent of flowering was rather conspicuous. It is not always seen to such an extent. The scion parent trees were also in very profuse flowering. The summer of 1940 was unusually dry and the trees were in their 'off' year that year. The heavy flowering of the parent trees and the scions taken from them were, therefore, considered to stand to reason.

Flowers appearing from such grafts in the nursery are normally removed. It was while removing them that the cases under report, i.e. flowers appearing from rootstock stems were discovered. On the whole, however, out of 269 Bombai, 628 Langra and 205 Fazli grafts, only 12, 1 and 7, respectively, produced panicles from their rootstock stems. In all these cases the scion shoots had also produced flowers.

\* Maintained jointly by the Imperial Council of Agricultural Research, India, and the Government of Bihar in the Department of Agriculture

In four of the Bombai and two Fazli grafts the flowers appearing from root-stock stem also set fruit (Plate XVIII, figs. 1, 2).

Mango grafts have been prepared in large numbers for over thirty years at Sabour but this is the first occasion when the phenomenon in question has been noted. It is possible that nursery men and research workers elsewhere have noticed this phenomenon some time or other but it is by no means of common occurrence.

An elucidation of the factors that induced flowering from rootstock stems of such grafts, would appear to be of great interest and importance in connection with studies on factors governing fruit-bud formation. It may be thought that lopping the rootstock stem above the graft union and of cincturing caused by the grafting bandage had an effect. These two factors are, however, always in association with grafting, but the phenomenon in question appears to be only occasional. It is also known that the mango tree can be made to flower by means of smudging at any time of the year, provided that the tree is in condition for forcing [Gonzalez, 1923]. Smudging can not, however, induce flowering if the shoot bud will not form reproductive organs [Alcala and Pedro, 1935]. In the present cases, however, the question of smudging does not arise as the grafts were not in any way subjected to such stimulation. The writer suggests that it is not unlikely that the scion had exerted some influence on the rootstock in some way or other. Here the scion parent trees were in a very favourable condition for fruit bud formation during the period of grafting. After inarching, when the union of the seedling and the scion shoot was effected, even when the latter was yet on the parent tree, metabolic translocation between the stock and the scion shoot was possible. Where the scion was in a very favourable condition for fruit bud formation, it is likely that the stock was also brought up to a similar level of such condition.

The observations are recorded here in the belief that they might be of interest to botanists and horticulturists.

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FIG. 1. A mango graft showing fruiting from rootstock stem



FIG. 2. A closer view of the graft shown in FIG. 1





# PLANT QUARANTINE NOTIFICATIONS

## INDIA

*Notification No. F. 193/40-A., dated 15th December 1941, issued by the Government of India in the Department of Education, Health and Lands*

**I**N exercise of the powers conferred by sub-section (1) of Section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government is pleased to direct that the following further amendments shall be made in the Order published with the notification of the Government of India in the Department of Education, Health and Lands, No. F. 193/40-A., dated the 3rd February 1941, namely :—

1. In clause (a) of paragraph 2 of the said Order—

(i) after the word “ permit ” the words “ in accordance with the form set forth in the Schedule to this Order ” shall be inserted, and

(ii) after the word “ behalf ” the following proviso shall be inserted, namely :—

‘ Provided that a permit shall not be refused in the case of any insect which, in healthy condition, is not likely to be destructive to crops ’.

2. To the said Order, the following Schedule shall be added, namely :—

### SCHEDULE

#### *Form of special permit authorising importation of insects*

1. Name, designation and full address of the importer.....
  2. Name of the insect species to be imported.....
  3. Stage or stages of the insect to be imported.....
  4. Country from which importation is sought.....
  5. Whether importation is intended by sea, land or air.....
  6. Whether in its original home it is a weed pest, a parasite or a predator.....
  7. (i) Name (names) of the weed (weeds) on which it is a pest in the country of origin.....
  - (ii) Name (names) of the pest (pests) on which it is a parasite or predator in the country of origin.....
  8. Name, designation and address of the exporter.....
  9. Quantity indented for.....
  10. Purpose of importation.....
- The above information is true to the best of my belief.

Date.....

(Signature of the importer).

I authorise the importation. This permit will be valid up to.....

Date.....

(Signature and designation of the  
certifying authority)

[N.B.—It is expected that the permit will be obtained in advance of sending the order so that the imported material may not remain indefinitely in the warehouse for want of suitable permit]

## ERRATA

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE, VOL. XII, PART I, FEBRUARY 1942

Page 15, line 23, from below, *for* ' Author ' *read* ' Another '

Page 22, line 19, *for* ' 30·53 0·41 ' *read* ' 30·53±0·41 '

Page 22, line 21, *for* ' 0°-2° ' *read* ' 30°±2° '

Page 22, line 23, *for* ' 941 ' *read* ' 1941 '

Page 26, Fig. 2, figures along abscissa, *for* ' 5°C., 10°C., 5°C., 0°C., 5°C., 0°C., 5°C., 0°C. ' *read* ' 5°C., 10°C., 15°C., 20°C., 25°C., 30°C., 35°C., 40°C. respectively '

Page 27, last line, *for* ' soil temperature ' *read* ' soil-temperature '

Page 28, line 1, *for* ' or ' *read* ' for '



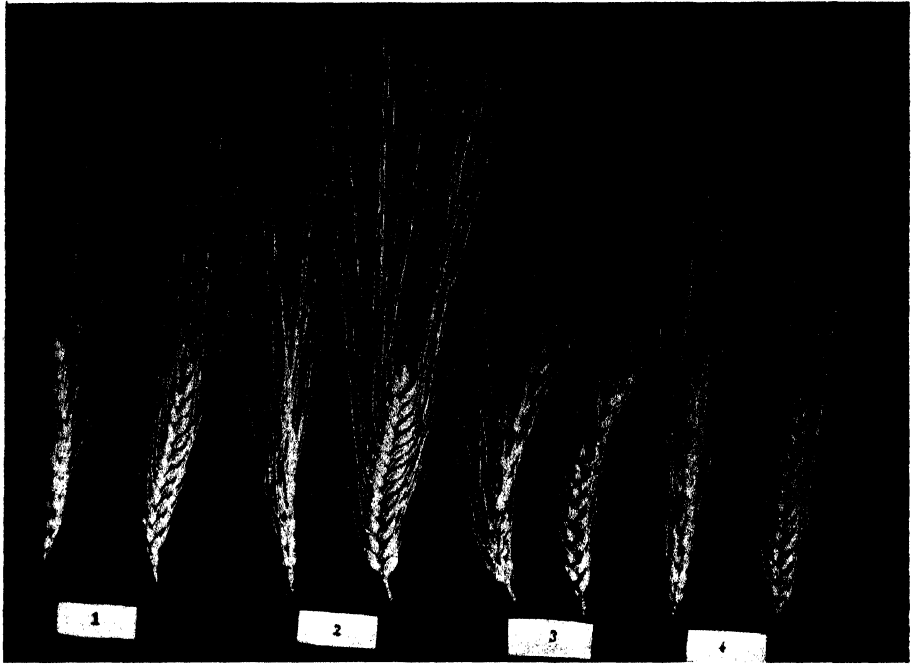


FIG. 1. Heads of 'blue' wheats (No. 1, 2, 4) and dwarf hill-wheat (No. 3)

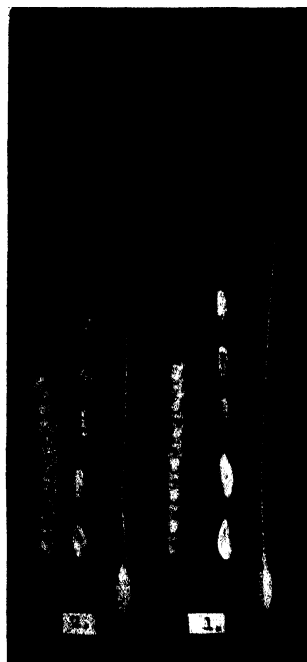


FIG. 2. Rachides, empty glumes, palea and kernels of 'blue' wheat (No. 1) and dwarf hill-wheat (No. 2) ( $\times \frac{2}{5}$ )

## ORIGINAL ARTICLES

### THE CYTOLOGY OF 'BLUE' WHEAT HYBRIDS

BY  
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AND  
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(Received for publication on 8 January 1942)

(With Plate XXVI and 21 text-figures)

**W**HEAT is the most popular crop in north China. It is next in importance to rice in the southern and south-western provinces of the Yangtze valley. Practically all of the important cultivated varieties belong to *T. vulgare*. The information regarding the existence of *Emmer* wheats and other species of *vulgare* group is rather meagre. As reported by various workers [Hosono, 1935, 1; King, 1934; Shen, 1937] varieties of *T. durum* have been grown in some localities in Yunnan, Hupeh, and Sinkiang and those of *T. turgidum* in Sinkiang, Honan and Kansu. It was not until our recent migration to Szechwan, after the Sino-Japanese hostilities, that we realized the significance of the so-called 'blue' wheat of Szechwan as a distinct group of *Emmer* wheats.

Many different local names are given to them and 'blue' wheat is the most popular and collective one. It is so named because the bloom or wax deposited on the surface of the green tissues appears bluish in colour. Another closely related form of *Emmer* wheats in Szechwan is known as dwarf hill-wheat. It differs primarily from the 'blue' wheat in the absence of waxy substance, in a lower plant stature, and in a somewhat different head type; yet both yield flours of extraordinarily poor quality. Information regarding the habitats of these wheats, as reported from various sources, seems conflicting. In some places they are suited to dry regions or mountain slopes, while in other places they grow well on moist, damp soils, such as river valleys. Therefore, opinions differ as to the botanic position of these wheats. Some state them to be *T. turgidum* and others *T. durum*. So far as the writers are aware, no cytological work on these two forms of *Emmer* wheats has yet been done. This paper reports an inquiry into the identification of these wheats by means of cytological studies on various 'blue' wheat hybrids.

#### GENERAL DESCRIPTION

**A. Geographical distribution.** 'Blue' wheat is found to be cultivated as a winter crop in various districts of western and north-western Szechwan. Its cultivation in this province goes back a long way and no exact evidence of the time of its introduction is available; but the limited degree of morphological and physiological differentiations or variations suggests that its introduction might not be of remote antiquity.

The dwarf hill-wheat, as indicated by the name, is commonly cultivated along the hillsides of the mountainous regions of western Szechwan. Although

no actual data concerning the acreage or production of these two forms of wheats in Szechwan is available, it has been estimated that they probably constitute about 5 per cent of Szechwan's wheat crop.

B. *General morphology.* In Szechwan where winters are mild 'blue' wheat (including dwarf hill-wheat) is customarily fall-sown. Within our collection there appear a number of varieties with different seedling habits. Among 249 strains of 'blue' wheat studied, 210 strains assume the upright, spring habit; 21 prostrate, and 18 semi-prostrate type. All the nine strains of dwarf hill-wheat possess upright seedlings. They seldom stool or tiller. The number of tillers varies from four to seven per plant. The leaves are usually broad and smooth but possess a peculiar whitish green colour with an extremely harsh cuticle. As a rule, the culms are somewhat taller (except dwarf hill-wheat) than those of common wheats, ranging from 0.915 to 1.515 mm., and hence the tonnage of green material produced per unit area tends to be great. This is, perhaps, one of the reasons why in some localities in Szechwan it is preferred for hay. The straw is of medium stiffness, with a dull, thick, striate surface.

So far we have not observed any 'blue' wheat which is beardless. The ears average 9 cm. in length and 1.5 cm. in width, possessing 20 or more spikelets. Three to five florets are found in each spikelet and generally three are fertile. Most varieties have short, thick, compact heads which are laterally compressed and more or less rectangular in cross section. The rachis is tough, smooth, but copiously fringed along its edges with white hairs and bears a frontal tuft of similar hairs at the base of each spikelet, reaching approximately a length of 3-4 mm. The empty glumes are white, pubescent on both surfaces and prominently and sharply keeled at the base. The awns are stout, rough, grayish white in colour, triangular in section, erect and projecting upward.

The grains are usually amber or yellow, but occasionally pale red in colour, large, broad and plump with a high dorsal arch or hump behind the embryo. The endosperm is opaque and starchy although in a few varieties it is quite hard and vitreous. A majority of varieties possess an intermediate condition, the texture is rather hard while opaquely white and non-translucent assuming a porcelain-like structure rather than starchy fractures. However, when they are grown under more or less humid conditions they are almost starchy.

As already pointed out above, 'blue' wheat is characterized by its waxy appearance. All of our collection except nine are waxy; the bloom covers all the plant parts. Those possessing no waxy bloom (here designated as 'dwarf hill-wheat') have very short, thick-walled culms with light, yellowish green foliage. The ears are fully bearded, with medium length and density. With the exception of the ear type, the morphological characteristics and the growth habit suggest that the so-called dwarf hill-wheat is most probably a related form of *T. pyramidale*.

C. *Cultural characteristics, quality and disease reactions.* As 'blue' wheat tillers less than the common winter wheat, seeding is, therefore, somewhat heavier than for common wheats. It grows rapidly and the heavily, fully bearded heads tend to have a nodding habit; this together with the

typically bloomy appearance makes them look like a fish tail and thus the name 'fish-tail' wheat is given by the farmers in certain localities.

The vigorous vegetative growth is accompanied by a delay of development. It heads about a week later than the late varieties of common winter wheats and consequently matures later. Such an inherently long period of growth should, in part, account for its high yielding capacity. But its tall growth and late ripening may be more readily subject to attacks by birds than the ordinary wheat. The straw is moderately stiff so that it is possible for birds to perch upon it and, because it is tall, it ranges above all other wheats and is more conspicuous to the eyes of the birds. It requires a moderately dry, hot season for satisfactory growth and thus it is more drought-tolerant. The hardness of the kernels is conditioned by the environmental factors.

As shown in our data on nitrogen determination (Table I) it may be said in general that 'blue' wheat tends to contain more protein than other wheats commonly cultivated in Szechwan, especially when the environment is favourable. Yet the baking quality of 'blue' wheat and dwarf hill-wheat flours is rather poor. Their gluten is less elastic than that of common wheats and their swelling capacity and the gas-retention capacity are unexpectedly low. They yield a heavier and stiffer dough with comparatively less water than other flours. Probably the gluten of 'blue' wheat as well as its starch differs physico-chemically from that of other wheats. This is the reason why flour from 'blue' wheat is primarily used in the baking of cakes and biscuits.

TABLE I  
*Protein content of wheats*  
(1940-41 crop from the University Farm, Chengtu, China)

Varieties	Moisture (per cent)	Protein (per cent)
7136 ('blue' wheat)	15.99	11.79
7181 ('blue' wheat)	15.28	12.20
7274 ('blue' wheat)	16.86	9.37
7174 (dwarf hill-wheat)	17.32	10.89
*NK 2905 (common wheat)	14.62	10.60
*NK 4197A (common wheat)	15.51	10.20

\* Improved varieties of the University Farm

None of the 'blue' wheat strains tested escapes from the infection of stripe rust, *P. glumarum*, which is rather serious in the west Szechwan plain, yet none of them reaches a severe stage of infection. They are more or less susceptible to loose smut, *U. tritici*, in most localities and, stinking smut, *T. tritici*, in certain districts. Scab, caused by *Gibberella saubinetii*, is of rare occurrence in Szechwan but, according to the writers' experience, these 'blue' wheats when sown in Kweichow, (a neighbouring province south of Szechwan) where wheats are generally harvested one month later than in Szechwan, suffer rather seriously from the attack of scab fungus. It is suggested that the compact spike of 'blue' wheat with heavy tufts of hairs at each rachis joint slows down the rate of drying after rains or heavy dews and consequently facilitates the attack of the pathogene. Wu [1940] tested

for the resistance of 'blue' wheat varieties to flag smut, *Urocystis tritici*, by artificial inoculation and found that none of the varieties tested was not immune. They are generally more susceptible, particularly those of soft varieties, to damage by flour moth and grain weevil than the common bread wheats under storage conditions.

Cytological observations reveal that the typical 'blue' wheat as well as the dwarf hill-wheat possesses normally 14 bivalents in meiosis (Fig. 1). The peculiarities of 'blue' wheat as a district group and its doubtful botanic position appear to justify a study of these subjects on which little literature is available. The present discussion rests primarily upon as yet incomplete information on the cytological behaviours of the hybrids between 'blue' wheat and other known species of *Triticum*.

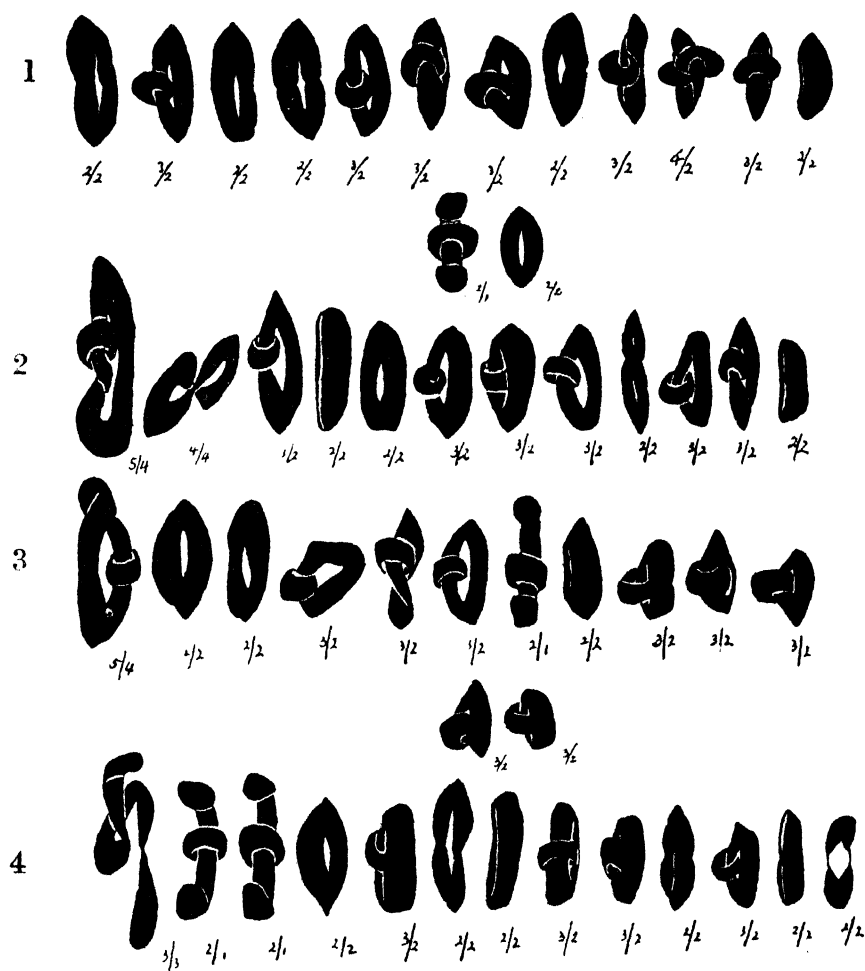
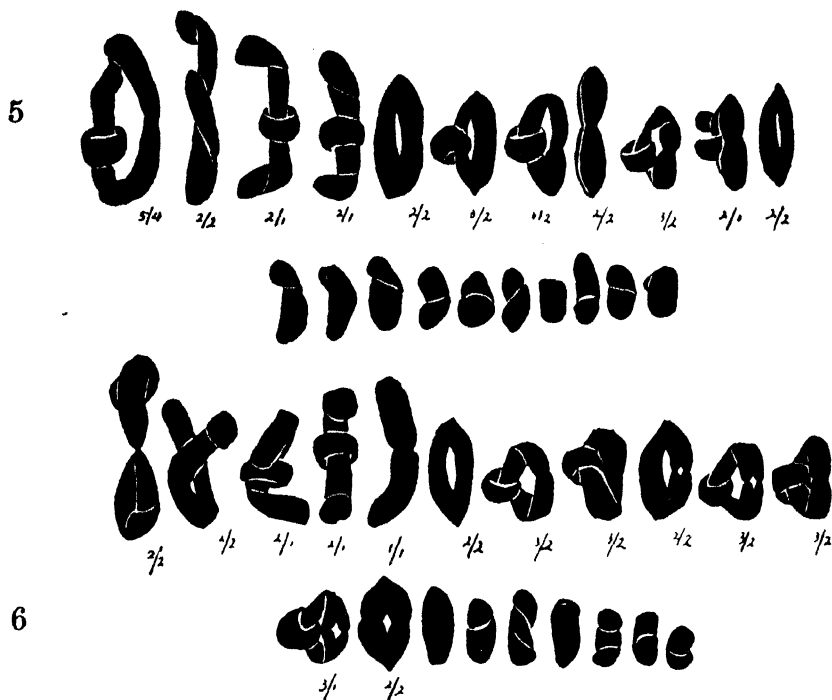


FIG. 1. Chromosomes of metaphase I in 'blue' wheat showing 14 bivalents

FIG. 2. Two rings of 4 and 10 bivalents in *T. durum* × 'blue' wheat

FIG. 3. A ring of 4 and 12 bivalents in *T. turgidum* × 'blue' wheat

FIG. 4. A chain of 4 and 12 bivalents in *T. pyramidale* × 'dwarf hill-wheat'



7(a)



FIG. 5. Metaphase I in *T. vulgare* × 'blue' wheat showing  
1 (IV) + 1 (III) + 9 (II) + 10 (I)

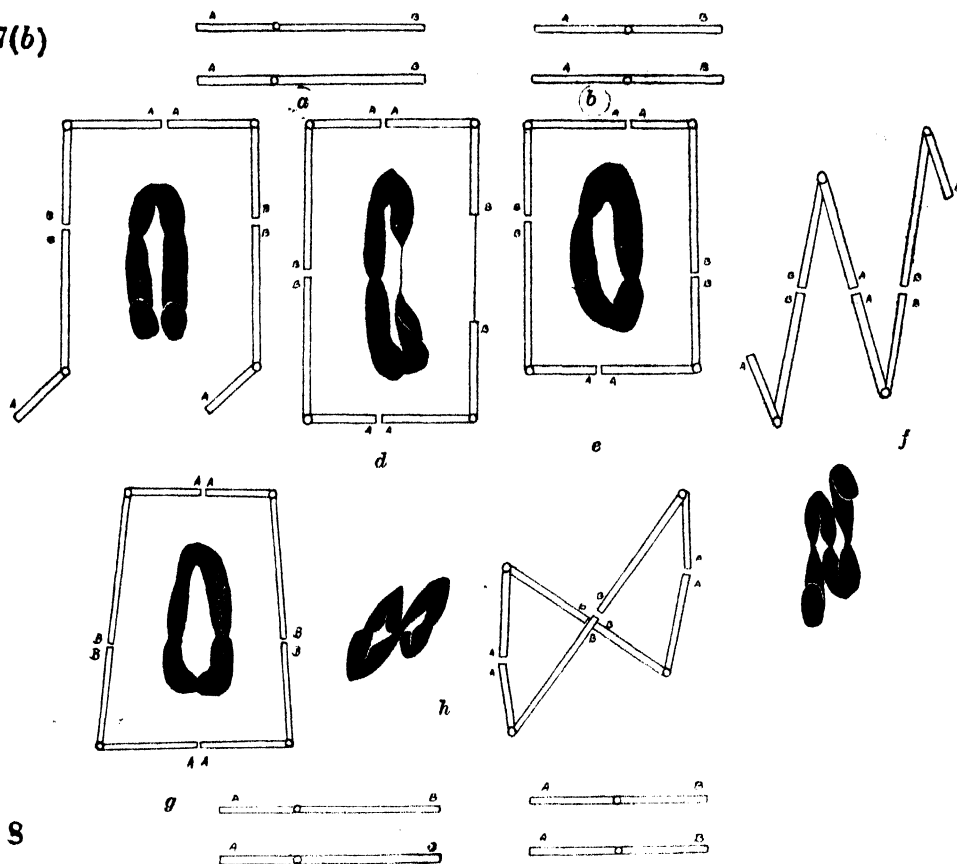
FIG. 6. Metaphase I in *T. sphaerococcum* × 'blue' wheat showing  
2 (III) + 11 (II) + 7 (I)

FIG. 7 (a). Association of 4 in *T. durum* × 'blue' wheat. All the four  
members possess sub-median centromeres

#### MATERIAL AND METHODS

The material used in the crosses includes *T. durum* Desf. (var. Iumillo) *T. turgidum* L., *T. pyramidale* var. *recognitum* Perc., *T. vulgare* (NK 2905) Host., *T. sphaerococcum* Perc. and 'blue' wheat and dwarf hill-wheat collected in Szechwan.

7(b)



8

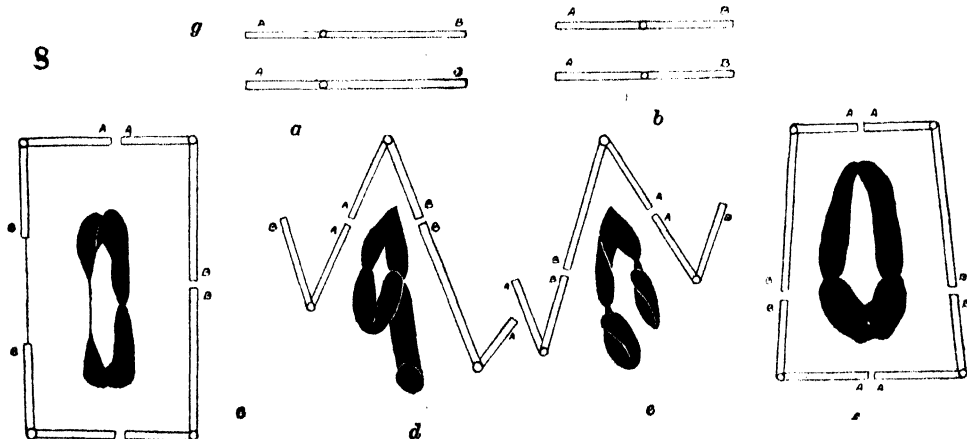


FIG. 7 (b). Configurations of *T. durum* × 'blue' wheat; a and b, two pairs of diagrammatic chromosomes taking part in the multiple configurations, one pair with median centromeres and the other pair with sub-median centromeres; c—h, associations of four found from different cells. They are composed of the same chromosomes

FIG. 8. Configurations of *T. turgidum* × 'blue' wheat; a and b, two pairs of diagrammatic chromosomes taking part in the multiple configurations, one pair with median centromeres and the other pair with sub-median centromeres; c—f, multiple associations found from different cells. They are composed of the same chromosomes. The two sets of four chromosomes in the two hybrids are morphologically similar

The crosses were made in 1940 and the slides were prepared in 1941. Smears were fixed in La Cour 2BE and were stained by Newton's gentian violet iodine method. Drawings were made with the aid of camera lucida.

## RESULTS

### A. Tetraploid hybrids

1. *T. durum* × B. W., ('blue' wheat). Besides the bivalents there are two rings of four chromosomes in this hybrid. One of the rings is composed of four chromosomes with sub-median centromeres (Fig. 7a), and the other comprises two chromosomes with median centromeres and two with sub-median centromeres (Figs. 2 and 7b). The average number of associations of four is 0.9 (Table II).

Bridges (Fig. 9) are observed in the first anaphase. The percentage of bridges is equal to 4.88 and the coefficient of hybridity is 0.00161.

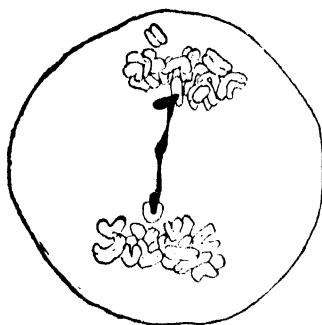


FIG. 9. First anaphase bridge in *T. durum* × 'blue' wheat

TABLE II  
Configurations of tetraploid hybrids






Configurations and No. of X-t a	Bivalents					Multiple configurations				Number of cells observed
						Association of four			Asso- ciation of 3+1	
	4.2	3.2	2.2	2.1	1.1	5.4	4.4	3.3		
<i>Durum</i> × B. W. (per cent)	..	54	182	1	9	2	11	4	..	20
	..	21.7	74.2	0.4	3.7	..	..	..	..	..
<i>Turgidum</i> × B. W. (per cent)	4	119	78	3	..	1	1	..	8	16
	2.0	58.3	38.2	1.5	..	..	..	..	..	..
<i>Pyramidalis</i> × D. H. W. (per cent)	..	74	38	5	5	..	4	5	..	10
	..	60.6	31.1	4.1	4.1	..	..	..	..	..

TABLE III  
Distribution of chiasmata of tetraploid hybrids

Hybrids	Mean No. of bivalent	X-ta per bivalent	X-ta per potential bivalent	Total X-ta per cell	Terminal X-ta per cell	Coefficient of terminalization
<i>Durum</i> × B. W.	12.30	2.18	2.15	30.15	27.30	0.905
<i>Turgidum</i> × B. W.	12.75	2.62	2.50	35.00	26.81	0.766
<i>Pyramidale</i> × B. W.	12.20	2.57	2.46	34.40	26.50	0.770

The mean number of bivalents per cell is 12.30. The number of chiasmata per bivalent is 2.18 and that for each potential bivalent is 2.15. The mean number of chiasmata is 30.15 and the coefficient of terminalization, 0.905.

2. *T. turgidum* × B. W. There is only one multiple configuration which is most frequently an association of three and occasionally an association of four (Table II; Figs. 3 and 8). In the latter there are two chromosomes possessing median centromeres and two possessing sub-median centromeres.

Both the first and the second anaphase of this hybrid are normal. The average number of bivalents per cell is 12.75. The mean frequency of chiasmata per bivalent is 2.62 and that for each potential bivalent, 2.50. The mean frequency of chiasmata per cell is 35.00. The coefficient of terminalization is 0.766.

3. *T. pyramidale* × D. H. W. (dwarf hill-wheat).\* There is only one association of four chromosomes (Fig. 4). The number of chiasmata per potential bivalent is 2.46 and that for each individual cell is 34.40. Out of 48 first divisions there are two single bridges (Fig. 11) in the same cell and five single bridges (Fig. 10) in 146 second divisions forming a percentage of 3.61. The coefficient of hybridity is 0.00105 showing that dwarf hill-wheat differs from *T. pyramidale* by a small portion of inversion.

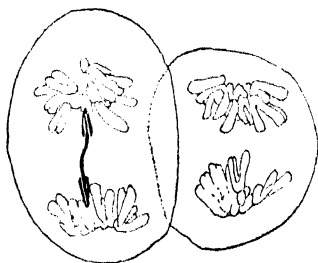


FIG. 10. Second anaphase bridge in *T. pyramidale* × dwarf hill-wheat

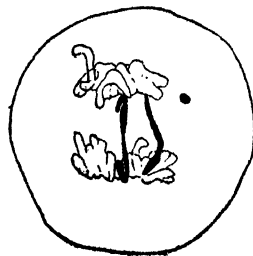


FIG. 11. Two single first anaphase bridges in *T. pyramidale* × dwarf hill-wheat

\*. The cross was made by C. K. Chan, to whom the writers are indebted

**B. Pentaploid hybrids**

1. *T. vulgare* (NK 2905)  $\times$  B. W. There are two multiple configurations, an association of four and an association of three (Tables IV and V; Fig. 5). The average number of bivalents per cell is 11.9 and the mean number of univalents per cell is 8.8. The average number of chiasmata per potential bivalent and that for each cell are 2.14 and 30.40 respectively. The coefficient of terminalization is 0.77. The percentage of bridges in the first division (Figs. 12 and 13) is 8.33 and the coefficient of hybridity is 0.00278.

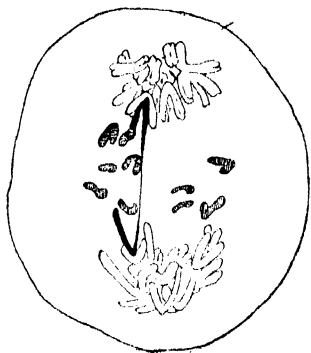


FIG. 12. First anaphase bridge in *T. vulgare*  $\times$  'blue' wheat

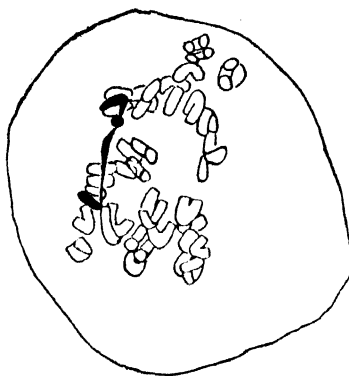


FIG. 13. First anaphase bridge and a fragment in *T. vulgare*  $\times$  'blue' wheat

TABLE IV

*Configurations of pentaploid hybrids*

Configurations and No. of X-ta	Bivalents				Multiple configurations				No. of cells observed
	4	3	2	1	Association of four			Association of three	
					5	4	3		
<i>Vulgare</i> × B. W.	2	58	43	16	1	1	1	4	10
<i>Sphaerococcum</i> × B. W.	2	20	31	5	..	1	1	3	5

TABLE V

*Distribution of chiasmata of pentaploid hybrids*

Hybrids	Average No. of bivalents per cell	Average No. of univalents per cell	X-ta per potential bivalent	X-ta per cell	Coefficient of terminalization
<i>Vulgare</i> $\times$ B. W.	11.9	8.8	2.14	30.40	0.77
<i>Sphaerococcum</i> $\times$ B. W.	11.6	8.4	2.11	29.60	0.68

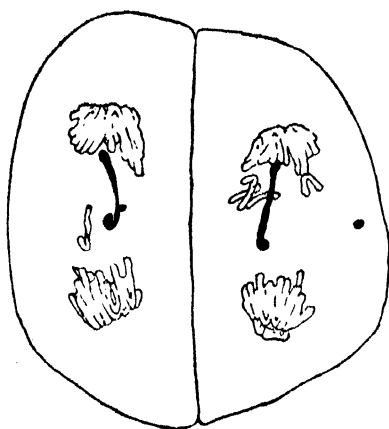


FIG. 14. Second anaphase bridges and fragments in *T. Sphaerococcum*  $\times$  'blue' wheat

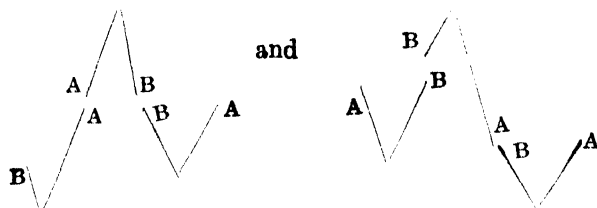
2. *T. sphaerococcum*  $\times$  B. W. Two associations of three (Fig. 6) are found in the same cell. The average number of univalents is 8.4 (Table V). The number of chiasmata per potential bivalent and that for each individual cell are 2.11 and 29.60 respectively. The coefficient of terminalization is 0.68. The coefficient of hybridity evidenced by the occurrence of bridges (Fig. 14) is 0.00135.

## DISCUSSION

### A. Configurations

1. *Tetraploid hybrids*. The 17 multiple configurations found in 20 P. M. Cs. of *T. durum*  $\times$  B. W. are exclusively associations of four. More than 76 per cent of the latter are ring-shaped and the remainder are chains of four either in the shape of N or of an open ring.

In *T. turgidum*  $\times$  B. W. the multivalents are most frequently associations of three. The one which fails to pair is always the one of the two with submedian centromere. For the association of three only two combinations are common, i.e.



In *T. pyramidale*  $\times$  D. H-W. the multiple configuration is always an association of four. Among 10 cells studied only one is devoid of such an association of four, instead of which two bivalents are present.

2. *Pentaploid hybrids*. In *T. vulgare*  $\times$  B. W. only two cells possessing 14 (II)+7 (I) are found. Among the six cells possessing multiple configurations four show apparent reduction of chiasma frequency as well as increase in the number of univalents ; showing that the formation of multiple configurations

must have something to do with the decrease in the number of chiasma frequency and the increase in the number of univalents. These will be dealt with in later paragraphs.

In *T. sphaerococcum* × B. W. none of the five cells examined show more than 13 bivalents. The combinations of the configurations are mostly 13 (II) + 9 (I), 1 (IV) + 12 (II) + 7 (I), 1 (III) + 11 (II) + 10 (I), 2 (III) + 11 (II) + 7 (I), and 1 (IV) + 11 (II) + 9 (I). The maximum number of associations of three is two and in the same cell the number of univalents is seven showing that there is intrahaploid pairing owing to external interchanges.

The formation of multiple configurations in the hybrids mentioned in this paper is all due to external interchange and this had been proved in the following ways :—

(a) Formation of bivalents in haploid plants of wheats—thus the number of bivalents was found to be one in *T. monococcum* [Kihara and Katayama, 1933 ; Chizaki, 1934], three in *T. durum* [Kihara, 1936]. In *T. vulgare* the number of bivalents was found to be one to two [Gaines and Aase, 1926], three [Yamamoto, 1936], four [Yamasaki, 1936] and nine [Krishnaswamy, 1939]. Krishnaswamy even found an association of three.

(b) Multiple configuration in pure species—thus in *T. turgidum*, Darlington [1931, 1] found an association of four.

(c) Intrahaploid pairing in intergeneric hybridization—in intergeneric hybrids a part of the bivalents is due to intrahaploid chromosome pairing in *Triticum*. Bivalents were found in *T. turgidum* × *Haynaldia villosa* [Berg, 1934], and in the hybrids between *T. turgidum* and species of *Aegilops* [Percival, 1930].

*T. durum* showed bivalents on hybridization with *Ae. ventricosa* [Katayama 1931 ; Matsumoto, 1933] and with *Secale cereale* [Kagawa and Chizaki, 1934 ; Plotnikowa, 1930].

Bivalents were also found in *Ae. ovata* × *T. vulgare* [Kattermann, 1932], *T. vulgare* × *Secale cereale* [Aase, 1930] and *Ae. ovata* × *T. sphaerococcum* [Percival, 1930].

(d) Intra or intergenomic pairing in intragenetic hybridization—Thompson [1926] found no multiple configurations in *T. monococcum* × *T. turgidum*. In *T. durum* × *T. monococcum* only a trace of multiple association was found [Aase, 1930]. In *T. vulgare* × *T. monococcum* four to twelve bivalents were found [Kostoff, 1935]. The extra five bivalents besides the normal seven are due probably to pairing between chromosomes of *vulgare*. The formation of numerous extra bivalents in *T. vulgare* × *T. monococcum* together with the rare occurrence of multiple configurations in *T. durum* × *T. monococcum* evidently shows that there are interchanges of chromosome segments between chromosomes of the same or of different genomes.

Contrary to the above findings, some previous investigators found no multiple configurations in *T. turgidum* × *T. vulgare* [Watkins, 1924], *T. sphaerococcum* × *T. turgidum* [Vakar, 1932] and *T. turgidum* × *T. durum* [Hosono, 1935]. The occurrence of multiple configurations in *T. vulgare* × B. W., *T. sphaerococcum* × B. W., *T. durum* × B. W., and *T. turgidum* × B. W. shows that the external interchanges of the chromosomes of 'blue' wheat are long enough for autosyndesis to take place.

The percentage of ring is much higher in *T. durum*  $\times$  B. W. than that obtained in tomato [Upcott, 1935]. In all the hybrids, the multiple configurations are almost exclusively rings and chains. Interstitial chiasmata are formed only between homologous members (Figs. 2, 3 and 5). Multiple configurations other than ring or chain require more than one chiasma on the same arm [Upcott, 1935], the failure of the latter indicates that the external interchanges between non-homologous chromosomes form terminal chiasmata only [Upcott, 1937, 2] and thus the duplications are not long enough for two chiasmata to be formed.

Morphologically the multiple configuration in *T. turgidum*  $\times$  B. W. is similar to one of the two in *T. durum*  $\times$  B. W. (Figs. 7 and 8). The constant similarity in the morphology of the multiple configurations of both hybrids suggests that the two sets of four chromosomes may be the same. If this is the case, then *T. durum* has two more common segments on those four chromosomes which form another association of four (Fig. 2) with 'blue' wheat than *T. turgidum* with 'blue' wheat.

The frequent failure of the association of four chromosomes in *T. turgidum*  $\times$  B. W. is due probably to the lower homology between the non-homologous members, as for the two types of configurations obtained there is only one fourth of chance that the fourth one can pair with its homologue.

The above mentioned shows that the homology of the homologous chromosomes is higher in *T. turgidum* and 'blue' wheat than in *T. durum* and 'blue' wheat; the homology between the non-homologous members is inversely true for the two hybrids.

In *T. pyramidale*  $\times$  dwarf hill-wheat, the interchanges are not only short, terminal ones but are limited to two pairs of chromosomes. All the other chromosomes show normal pairing, except the inverted regions.

### *B. Interference of pairing between configurations*

Among the 10 cells mentioned in *T. vulgare*  $\times$  B. W., there are four cases wherein association of more than two chromosomes is correlated with an increase in the number of univalents as well as a decrease in the frequency of chiasmata. It seems likely that chiasma formation between more than two chromosomes interferes with the chiasma formation of the other members. It is due probably to interference of pairing during the zygotene stage [Darlington, 1937]. This coincides with the finding in *Matthiola incana* by Armstrong and Huskins [1934] that the increase of multiple associations resulting from translocations and duplications is correlated with the decrease in normal pairing. The frequencies of the multiple configurations, the univalents and the chiasmata for individual cells are listed in Table VI.

The relation between the multiple configuration and the total chiasma frequency for every individual cell is detected by computing the coefficient of contingency between the two factors mentioned. The 'C' value and its standard error are  $0.992 \pm 0.005$ , showing that multiple configurations are negatively correlated with the frequency of chiasmata. The reduction in the chiasma frequency is undoubtedly due to interference in normal pairing which is a consequence of the formation of multiple configurations.

TABLE VI

*Distribution of multiple configurations, univalents and chiasmata*

Cells	1	2	3	4	5	6	7	8	9	10
Multiple config.	1	0	0	1	0	2	1	0	1	1
Univalents	5	11	7	10	7	10	13	7	12	6
Chiasmata	36	30	32	26	30	27	25	37	29	32

Ribbands [1937] in *Lilium*  $\times$  *testaceum* found that there was no relation between the frequency of univalents and the chiasma frequency of the remaining bivalents. The writers' results show that the frequency of univalents is negatively correlated with the chiasma frequency. The value of 'rank correlation' and its standard error amounting to  $-0.755 \pm 0.136$  show that the frequency of univalents is a net index of the failure of pairing which is a consequence of interference.

Thus the interference caused by the formation of multiple configurations can be detected by either the chiasma frequency or by the frequency of the univalents. The frequency of univalents is positively correlated with the frequency of multiple configurations and the chiasma frequency is negatively correlated with the latter.

Interference between configurations other than the multiple configurations [Mather, 1936] cannot be detected because the separate bivalents cannot be distinguished.

### C. Structural changes

1. *Inversions.* Bridges of the first division are due to crossing-over in the inverted region which results in an acentric fragment and a dicentric chromatid. Bridges in the second division are due to crossing-over in the inversion which results in a loop and an acentric fragment in the first division and thus bridge and fragment in the second division [Darlington, 1937].

(a) Number of inversion: The occurrence of two single bridges in one cell in *T. pyramidale*  $\times$  B. W. indicates that there are two chromosomes possessing inversion. In all the other hybrids except *T. vulgare*  $\times$  'blue' wheat only one chromosome possesses such an inversion.

(b) Size of inversion: The size of the acentric fragment equals the sum of the length of the inversion and the portion distal to the inversion [Upcott, 1937, 2]. The small size of the fragment (Figs. 11, 13 and 14) shows that the size of the inversion can hardly be large.

(c) Position of inversion: In *T. durum*  $\times$  B. W. the bridge is quite thick and the arms of the bridge are only a little bit shorter than the thick portion of the bridge (Fig. 9). Thus the container of the inversion and its homologue

probably have sub-median centromeres and the inversion is near to the end of the longer arm [Richardson, 1936 ; Upcott, 1937, 2]. This is supported by the small size of the fragment. The chromosomes involved in *T. durum* × B. W. and *T. vulgare* × B. W. are probably similar members (Figs. 9 and 13). Another bridge (Fig. 12) from a different cell of *T. vulgare* × B. W. shows a different morphology. The chromosomes probably have median centromeres. If this is so, then *T. vulgare* × B. W. is heterozygous for at least two inversions. In *T. sphaerococcum* × B. W. the inverted segment is near to the end of the longer arm of the chromosomes possessing subterminal centromeres (Fig. 14). It seems likely that the container of the inversion and its homologue are a different pair of chromosomes in comparison with *T. durum* × B. W. and *T. vulgare* × B. W. The two bridges are due to the two loops and two fragments of the first division resulting from a chiasma proximal to the inversion, which is disparate with respect to complementary chiasmata in the inversion.

In *T. pyramidale* × dwarf hill-wheat the inverted segment is on the shorter arm, because the bridge is thin and the arms are, on the contrary, long and thick (Fig. 10) [Richardson, 1936]. The other chromosome possessing an inversion has sub-median centromere (Fig. 11).

The striking thing to which attention should be paid is that so far as our material is concerned no bridge formation occurs in *T. turgidum* × B. W. This logically follows that *T. durum* but not *T. turgidum* differs from 'blue' wheat by an inversion. Here again, a higher degree of homology is revealed between chromosomes of *T. turgidum* and 'blue' wheat than those of *T. durum* and 'blue' wheat.

2. *External interchanges.* The details are mentioned under 'A. Configurations'.

3. *Undefined structural changes.* The undefined structural changes result in the reduction of the frequency of chiasmata [Darlington, 1937]. The general difference of the chromosomes is shown in Tables II to V. The higher degree of homology between the homologous chromosomes of *T. turgidum* and 'blue' wheat not only gives 0.45 more bivalents (in comparison with *durum* × B. W.) but also shows a higher frequency of chiasmata for every individual bivalent, every potential bivalent and a higher average total frequency of chiasmata per cell. In *T. turgidum* × B. W. 76.6 per cent of the total chiasmata is terminal as compared with 90.5 per cent in *T. durum* × B. W., in other words, 23.4 per cent in the former and 9.5 per cent in the latter are interstitial. Since a higher frequency of chiasmata, especially that of interstitial ones, is always positively associated with the degree of affinity, therefore, we have another evidence that phylogenetically 'blue' wheat is more closely related to *T. turgidum* than to *T. durum*.

The frequency of chiasmata in *T. pyramidale* × D. H. W. is nearly as high as that in *T. turgidum* × B. W. (Table III). It shows not only a similar distribution, but also a very close mean as compared with that in *T. turgidum* × B. W. A comparison of the bivalents possessing different number of chiasmata is shown in Fig. 15, and the distribution of total chiasmata for individual cells is listed in Table VII.

Both the frequency of chiasmata per potential bivalent and that per cell in *T. vulgare* × B. W. are similar to those in *T. sphaerococcum* × B. W.; but in

the latter the coefficient of terminalization is somewhat lower. The distribution of total chiasmata for individual cells of the pentaploid hybrids is listed in Table VII.

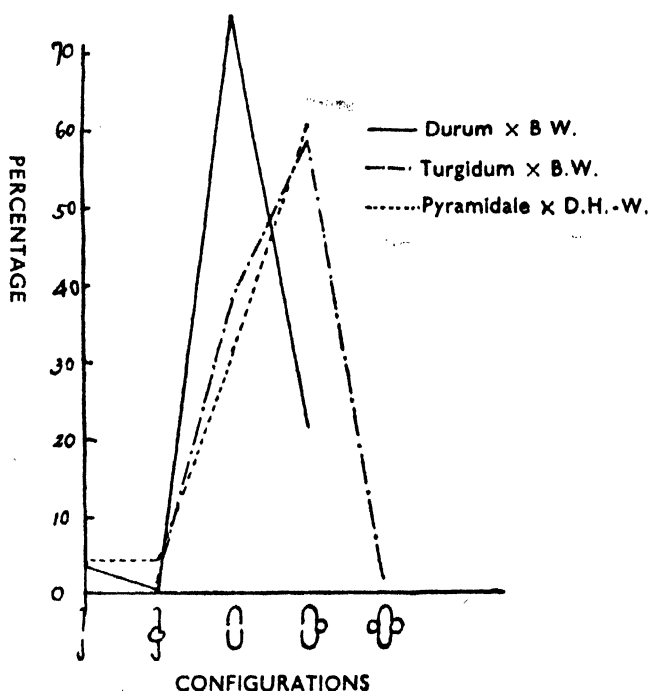


FIG. 15. Showing the comparison of the bivalents possessing different number of chiasmata

TABLE VII  
*Distribution of total chiasmata for individual cells*

Chiasmata	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	No. of cells observed	Mean & S. D. m
<i>Durum</i> × B. W.		1		3	4	4	4	2		1	1						20	30.15 ± 0.460
<i>Turgidum</i> × B. W.							1	2	3	3		1	2	3		1	16	35.00 ± 0.647
<i>Pyramidale</i> × B. W.							2	2			2	1	2	1			10	34.40 ± 0.812
<i>Vulgare</i> × B. W.	1	1	1		1	2		2				1	1				10	30.40 ± 0.194
<i>Sphaerococcum</i> × B. W.		1		1		1		2									5	29.60 ± 0.043

#### D. Anaphase division

1. *Behaviours of univalents.* The univalents are distributed at random and usually divide in the first and lag in the second division. They form

supernumerary nuclei during the tetrad stage, if they are not included in the daughter nuclei.

In *T. pyramidale*  $\times$  D. H. W. the univalent laggard may fail to divide (Fig. 16) in the first division. There is no doubt of its being a true univalent [Upcott, 1937, 1]. Its failure to divide is due probably to a delay in the moving on to the equator [Darlington, 1937].

2. Fragmentation of univalents has been observed in the second anaphase of *T. sphaerococcum*  $\times$  B. W. (Figs. 17, 18 and 19), due probably to the mis-division of centromeres described by Upcott [1937, 1] and Darlington [1939]. Mis-division takes place in both divisions. A four-to-none type of division (Fig. 19) has been observed. They more frequently show normal division in the first anaphase and mis-divide in the second anaphase (Figs. 19 and 21). The mis-division of the centromere is due to the double structure of the latter [Darlington, 1939, 1940].



FIG. 16. Failure of division of a univalent lagged in anaphase I of *T. pyramidale*  $\times$  'blue' wheat

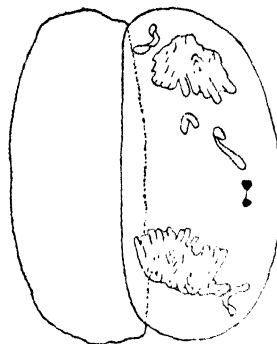


FIG. 17. Mis-division of a univalent in second division of *T. sphaerococcum*  $\times$  'blue' wheat

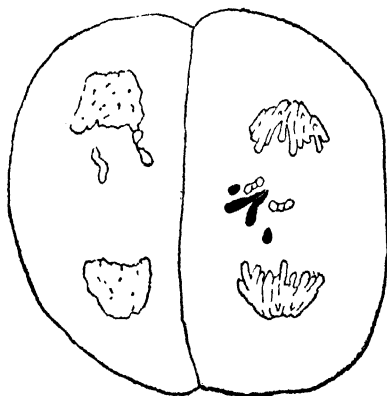


FIG. 18. Mis-division of univalent. A 4-0 type of division takes place in the first division leaving two centric and two acentric arms. Besides, there is, probably, a free centromere

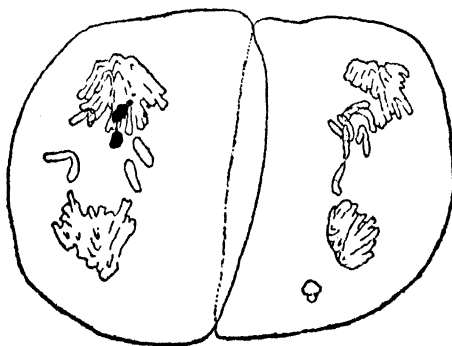


FIG. 19. A univalent mis-dividing in the second division

### *Non-disjunction of bivalents*

2. Non-disjunction of bivalents has frequently been observed in *T. durum*  $\times$  B.W. (Fig. 20). This is due to the presence of the interstitial chiasmata distal to which there is probably a change of homology which makes complete terminalization impossible. This is similar to the finding of Darlington [1931,2] in *Oenothera* and that of Philp and Huskins [1931] in *Matthiola*.

### *Formation of unbalanced gametes*

During the second metaphase of *T. pyramidale*  $\times$  B. W. a plate showing  $n = 16$  has been observed (Fig. 21). This is due, most probably, to non-disjunction of a multiple association of which the centromeres lie indifferently with one another.

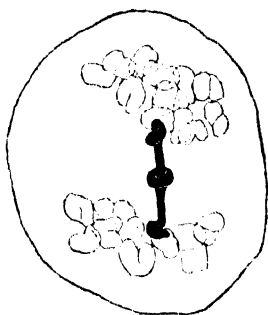


FIG. 20. Non-disjoined bivalent in *T. durum*  $\times$  'blue' wheat



FIG. 21. Metaphase II in *T. pyramidale*  $\times$  D. H. W., showing  $n = 16$

## SUMMARY

Judging from the multiple configurations, the occurrence of inversions and a comparison of the general frequency of chiasmata as well as the average number of bivalents, 'blue' wheat is phylogenetically nearer to *T. turgidum* than to *T. durum*.

The occurrence of only one association of four and the high frequency of chiasmata together with the absence of wax as well as the dwarf height of the plant which rarely exceeds 3 ft. reveal that dwarf hill-wheat might be related to *T. pyramidale*.

The pentaploid hybrids between *T. vulgare*, *T. sphaerococcum* and 'blue' wheat show two multiple configurations. The numbers of univalents are 8.8 for *T. vulgare*  $\times$  B. W. and 8.4 for *T. sphaerococcum*  $\times$  B. W.

Bridges are observed in all the hybrids except *T. turgidum*  $\times$  'blue' wheat.

The formation of multiple configuration is correlated with the decrease in normal pairing in *T. vulgare*  $\times$  'blue' wheat.

Non-disjunction of bivalent and formation of unbalanced gametes are observed in the tetraploid hybrids.

Fragmentation of univalents is observed in *T. sphaerococcum*  $\times$  'blue' wheat.

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# \*STUDIES IN THE PERIODIC PARTIAL FAILURES OF THE PUNJAB-AMERICAN COTTONS IN THE PUNJAB

## VII. AMELIORATION OF *TIRAK* ON SOILS WITH SALINE SUBSOILS (SANDY LOAMS)

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(With Plates XXVII and XXVIII)

IN one of the previous contributions [Dastur, 1941] the ameliorative effect on the opening and yields of the application of the sulphate of ammonia to cotton plants that developed *tirak* on account of a deficiency of nitrogen on light sandy soils was described. When the sulphate of ammonia was applied to such light sandy soils the premature yellowing and shedding of the leaves did not occur in the crop, the opening of bolls improved and the yield of *kapas* was greatly increased. A rapid method of spotting the deficiency of nitrogen in the crop called the 'tannin test' was also dealt with. It was equally important to develop remedial measures for amelioration of *tirak* on soils with saline subsoil and intensive studies were undertaken on this aspect of the problem.

It has already been pointed out [Dastur and Sucha Singh, 1942] that the nature of 'physiological disorder' that sets in plants growing on saline subsoils was different from that which occurs in plants on light sandy soils. In the latter case the remedial measure for such soils was simple after such soils were spotted. In the case of soils which have high salinity in the subsoil, the problem of removing or counteracting salinity at a depth of 3-4 ft. was rather difficult and complicated. The probable remedial measures for saline subsoils can be classified into three groups; (1) There are 'known' remedies which would counteract the toxic effects of sodium salts. Under this category may be mentioned the application of gypsum or any other calcium salt which would antagonize the toxic effects of free soluble sodium salts and which by a process of base exchange may replace the exchangeable sodium with calcium in the clay complex. The toxicity of sodium salts is known to depend on the physical texture of the soil. Higher percentages of

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clay or presence of organic matter in the soil may detoxicate the sodium salts. Application of organic manure like the farmyard manure or the green manure like berseem or additions of silt (a substitute for clay) were, therefore, regarded as possible remedies. The use of inorganic fertilizers like nitrogen, superphosphates and potash was also considered as they might not be available to the plants from such soils. (2) Salinity in the subsoil may be washed down from the feeding zones of the roots by flooding such fields. Flooding may be combined with application of gypsum. (3) Measures by which the occurrence of water deficit in the cotton plants at the reproductive stage can be either removed or avoided. This group would naturally include the extra applications of water to increase the amount of available water by keeping the non-saline upper surface moist and the reduction of the total leaf area of the plants by cutting down their vegetative growth.

The applications of these remedial measures become specially difficult on account of great heterogeneity that is prevalent in the Punjab soils. The intermingling of normal soils, saline soils and light sandy soils in the same field, presents difficulties for applications of substances to counteract the toxic effect of sodium salts or of washing them down to lower layers by flooding. Salinity occurs in such an irregular manner that such measures would have to be applied even where they were not required. As for instance flooding may wash down the salts from the feeding zones of the roots in those parts of a field where salinity is present in the subsoil but it would also leach the soil of important nutrients from normal non-saline areas, specially from the light sandy portions, and render them infertile. It is, therefore, necessary that any treatment that is either applied to the soil to counteract or to remove salinity in the subsoil must be such as to ameliorate the soil conditions in the saline areas while it does not at the same time in any way adversely affect either the soil conditions or the growth of plants in non-saline parts. If this point is not borne in mind the removal of one evil may be accompanied by the creeping in of another.

Another difficulty is that the treatments for counteracting the toxic effect of salinity must penetrate to a depth of 3 ft. or more in order to prove effective. That would render their use impracticable as well as uneconomical.

#### INVESTIGATION

The occurrence of *tirak* was noticed in the cotton season of 1936 in a field at the Lyallpur Agricultural Farm. The soil conditions and the behaviour of the plants growing on this field were studied, side by side. The positions of the normal and *tirak* patches were carefully marked and the soil conditions under normal and *tirak* crop were investigated. It was established that *tirak* had developed in regions where the subsoils were saline while the subsoils under normal crop were non-saline. This field measuring 138 ft.  $\times$  288 ft. was, therefore, particularly selected for the trial of the remedial measures of the type discussed above, in the cotton season of 1938.

The ameliorative treatments were : (1) flooding, (2) gypsum + flooding, (3) inorganic fertilizers (sulphate of ammonia + superphosphate) after flooding, (4) gypsum + flooding + inorganic fertilizers and (5) farmyard manure followed by flooding. The sixth treatment was control,

With the exception of inorganic fertilizers, the other applications were made far in advance of sowings. Gypsum was added in two doses of 500 lb. each per acre in December, 1937 and January, 1938 followed by flooding each time. Flooding was also started from December. Each time the land came in *wuttar* it was ploughed up. Flooding was done three times before watering for sowing was given. Farmyard manure was applied at the rate of 10 tons per acre. The sulphate of ammonia was applied at the rate of 50 lb. N per acre and the superphosphate at the rate of 150 lb.  $P_2O_5$  before sowing. Thus an attempt was made to include all the remedial measures of the first two categories.

The remedial measure of the third category was also tried. The idea was to cut down the leaf area by reducing the vegetative growth and the most natural way to accomplish this was to defer sowings by one month as compared with the normal sowings. As a preliminary trial the inclusion of two sowing dates as treatments was, therefore, considered desirable. The field was divided into four blocks. Each block consisted of two main plots for the two sowing date treatments. The main plots were subdivided into six sub-plots to which were assigned at random the six treatments enumerated above. It was, therefore, a split plot design with eight main plots consisting of 48 sub-plots of  $1/80$  acre each.

Thus it would be normally expected that the most precise information will be obtained on the six sub-plot treatments while the information that would be obtained on sowing dates will be less precise as they were allocated to the main plots. Unsown interstrips were kept in between sub-plots to avoid shading and seepage effects.

Sowings were done on 5 and 6 May, and 7 and 8 June in the early and the late sown plots respectively by dibbling as the small size of the sub-plots did not permit the use of a hand drill. Eight seeds per hole were dibbled at  $2\frac{1}{2}$  ft.  $\times$   $2\frac{1}{2}$  ft. distance. Each hole was equidistant from the six surrounding holes (equilateral system). On account of a heavy shower of rain on 12 June, received soon after germination, the late sown plots had to be resown on 15/16 June. Thus the two sowing dates under trial were 5/6 May and 15/16 June. The plants were finally thinned to two plants per hole. The early-sown crop received in all eight waterings, while the late sown received six waterings. With the exception of the first two irrigations to the early-sown crop, subsequent irrigations were given on the same day to both the sowings. The last date of watering for both the sowings was 18 October.

The crop in each plot was under close observation throughout the season. Nothing abnormal was noticed in the condition of the plants under each treatment uptil September when the May-sown cotton plants began to show drooping of leaves. The drooping of the leaves in the May-sown plants occurred irrespective of the six ameliorative treatments in all plots where the subsoil was saline while drooping of leaves was not noticed in any one of the plots which were sown in June. Thus one of the symptoms of 'disease' was markedly absent in the June-sown plots indicating that reduction in leaf area prevented the development of the water deficit that occurred on such soils. The June-sown plants were much smaller in size and consequently produced less foliage than the May-sown crop.

Drooping of leaves was followed by excessive defoliation of the May-sown plants while shedding was much less in the June-sown crop under similar conditions as compared with the early-sown crop at the same stage of morphological development of the plant. The leaves of the two sowings presented striking differences in colour and appearance at fruiting. They remained green and fresh in the June-sown plants and dull and blackish green in the May-sown plants.

At the time of flowering and fruiting the two sowings exhibited other important and distinctive features that deserve mention. A delay in sowing by about 40 days shifted forth the onset of flowering by about 12 days only. The flowers were mostly confined to the tips of the branches in the May-sown plants; the lowermost nodes of the main stem did not directly produce the fruiting branches which were located high up on the main stem and the secondary branches. In the June-sown plants the sympodia arose directly from the lower nodes on the main axis and the flowers did not, therefore, appear aggregated at the tips as they were widely spaced on them. The growth of the fruiting branches was more vigorous in the late-sown than in the early-sown crop and so the successive flowers were separated by longer internodes in the former (Plate XXVII, figs. 1 & 2).

The most reliable criterion to judge the quality of opening of the bolls is the weight of seed cotton per boll. The bolls that are badly opened (i.e. the bolls of *tirak* plants) contain immature seeds and poor lint and, therefore, the weight of seed cotton per boll falls. Contrariwise, the maturity of seed would be reflected in raising the weight of *kapas* of a boll and will be indicative of good opening. It was, therefore, undertaken to determine the effect of different treatments on the weight of seed cotton per boll. Two units of five pair of plants each were tagged at random in each sub-plot for this purpose. The number of opened bolls from these samples and weight of *kapas* produced by them were recorded before each picking. The yield of the experimental area in each sub-plot was then weighed and recorded.

Nodal counts and height measurements of the individual plants tagged for boll weight in each sub-plot were taken when pickings were over. Thereafter the number of sticks and their weight were determined plot by plot. These determinations would provide information on the growth made by the plants under two sowing dates and the six sub-plot treatments. With these records the weight of seed cotton produced by 100 gm. of stem dry matter can be computed to get an idea of the efficiency of the cotton plants for production of seed cotton under different treatments.

The data collected were subjected to statistical analysis. The tables for analyses of variances are given below (Table I). A summarized record showing the nature and magnitude of the effect of different treatments is presented in Table II. The differences between the two dates of sowing came out highly significant in all determinations despite inadequate replication while the sub-plot treatments did not differ significantly among themselves. The interaction of sowing dates with sub-plot treatments was non-significant indicating no differential behaviour of time of sowing with the sub-plot treatments.



FIG. 1. Early-sown (5 May) 4F P.-A. cotton plant (leaves removed) showing that flowers and bolls are borne near the tips of main stem and branches



FIG. 2. Late-sown (16 June) 4F P.-A. cotton plant (leaves removed) showing that flowers and bolls are not aggregated at the top, but are borne at the lower



FIG. 1. Badly opened bolls with lower leaves shed of the May-sown crop on soils which are saline in the subsoil



FIG. 2. Well-opened normal bolls of the late-sown crop on soils which are saline in the subsoil

TABLE I  
*Analyses of variances*

Due to	Yield in gm.		No. of bolls		Weight of seed cotton per boll		Height in cm.		Stem weight per plant gm.		Seed cotton per 100 gm. stem weight	
	D.F.	Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square
Blocks . . .	3	8934455.0	24.886**	7676.6	...	0.3787	..	502.1	..	9150.7	..	217.82
Dates . . .	1	148086172.6	398.411**	145782.1	148.96**	4.1251	23.119**	8284.1	15.81*	252735.2	53.77**	23306.86
Error (a) . . .	3	359016.3	..	981.8	..	0.1467	..	520.7	..	4098.9	..	105.43
Treatments . . .	5	630280.7	..	774.64	..	0.0194	..	42.1	..	1865.6	..	66.83
Dates × Treatments . . .	5	513032.6	..	1535.60	..	0.0680	..	200.9	..	2303.6	..	61.12
Error (b) . . .	30	1112926.1	..	2128.99	..	0.1374	..	127.8	..	1261.9	..	29.22

\*Significant at 5 per cent level of significance  
 \*\*Significant at 1 per cent level of significance

**TABLE II**  
*Treatment effects on the vegetative and the reproductive characters*  
**Experiment I**

	Control	Gypsum	Flood- ing	NP	Gypsum + NP	F. Y. M.	Means for sow- ing dates	Differ- ence	C. D. 1 per cent
<i>Average yields in lb. per plot</i>									
May-sown (D1)	7.5	8.3	9.2	8.2	7.8	7.5	8.1	6.0**	2.33
June-sown (D2)	15.3	14.2	14.7	16.4	15.4	13.6	14.1	...	...
Mean	11.4	11.2	12.0	12.3	11.6	10.5	...	...	...
<i>Average No. of bolls per hole</i>									
May-sown (D1)	25.7	28.9	31.4	25.7	31.2	26.2	28.2	15.6**	7.48
June-sown (D2)	42.6	43.5	42.1	47.7	44.0	42.7	43.8	...	...
Mean	34.2	36.2	36.8	36.7	37.6	34.4	...	...	...
† Sampling error per plot = 10.9 per cent of the mean									
<i>Average weight of seed cotton per boll in gm.</i>									
May-sown (D1)	1.89	1.95	1.95	1.78	1.86	1.87	1.88	0.42**	0.25
June-sown (D2)	2.35	2.20	2.32	2.42	2.29	2.21	2.30	...	...
Mean	2.12	2.07	2.13	2.10	2.07	2.04	...	...	...
Sampling error per plot = 5.9 per cent of the mean									
<i>Average height in cm. per plant</i>									
May-sown (D1)	101.6	109.6	109.4	103.4	100.6	104.8	104.8	18.5*	14.8***
June-sown (D2)	85.4	84.3	83.1	89.9	92.3	82.7	86.3	...	...
Mean	93.5	96.9	96.3	96.6	96.6	96.5	...	...	...
† Sampling error per plot = 3.4 per cent of the mean									
<i>Average weight of stem per hole</i>									
May-sown (D1)	283.3	341.5	334.5	353.8	311.0	294.5	310.3	144.7**	115.6
June-sown (D2)	181.3	152.5	162.0	180.0	189.0	173.0	174.6	...	...
Mean	232.3	247.0	248.0	266.9	255.0	233.8	...	...	...
<i>Average seed cotton per 100 gm. of stems</i>									
May-sown (D1)	19	16	19	16	17	19	17.6	42.9**	17.29
June-sown (D2)	60	67	64	65	51	56	60.5	...	...
Mean	39.5	41.5	41.5	40.5	34	37.5	...	...	...

\*Significant at 5 per cent level of significance

\*\*Significant at 1 per cent level of significance

\*\*\*C. D. at 5 per cent level of significance

† Sampling errors per plot for various determinations were of an order so as to justify that the samples drawn were fairly representative of the entire plots. The conclusions drawn were, therefore, applicable to the entire plots

‡ Low sampling error for height indicates that this character is less variable than boll number or weight of seed cotton per boll

The performance of deferred sowing was superior to May sowing in case of yields, boll numbers and the weight of seed cotton per boll. As already mentioned the late-sown plants did not exhibit the *tirak* symptoms, viz. drooping of leaves and their premature shedding. As the opening of the bolls in the late-sown crop was significantly superior to opening of bolls in the May-sown crop, the maturity of seeds in the bolls in the former case was greater than that of the latter. Apparently the mean value 1.88 gm. for

weight of seed cotton per boll is high for a *tirak* crop. But that was due to two reasons ; firstly, the field was fallow during the previous year and received a lot of preliminary tillage and, secondly, the soil was not uniformly saline in the subsoil over the entire field. Salinity in the subsoil was present in patches only, while non-saline areas were scattered about at random in-between portions having either low salinity or no salinity in the subsoil. The weight of seed cotton in the plots where the soil was saline had gone down to 1.0 gm. per boll but that was not the case with the late-sown plants under similar soil conditions. This difference was reflected in the average weight of seed cotton per boll in the June-sown crop.

Evidently the late sowing had reduced the vegetative growth as the height per plant and stem dry matter per plant were significantly lower in the late-sown plants than those attained by the May-sown.

The late sowing had definitely ameliorated *tirak* and the conception of reducing the leaf area of the plant so that water deficit in the crop may not arise was found to hold good.

The efficiency for production of seed cotton in the late-sown crop appeared to be significantly higher than that of the early-sown crop. The proportion of seed cotton produced per unit dry matter of stems was much higher in the late-sown than in early-sown plants. This is important as what was required was more of seed cotton rather than of the vegetative growth.

The ameliorative effects of similar treatments for counteracting the deleterious effects of salinity in the subsoils were again studied in the same field in 1939-40 cotton season. Sowing date was omitted from the experiment as its effect on *tirak* was studied in a separate experiment to be described later. This experiment was repeated with some changes in the nature of the treatments. The plots under flooding in the previous year were treated with canal silt at the rate of 80 tons per acre. The plots receiving farmyard manure in the previous year were green manured at the rate of 10 tons per acre. The treatment gypsum and inorganic fertilizers of 1938-39 was substituted by green manuring supplemented by the same inorganic fertilizers, the latter given as a split application in two equal doses (half at sowing and half at flowering, total quantities added being the same as in the previous experiment). The remaining three treatments were the same, viz. gypsum, nitrogen and phosphorous, and control and they were allotted to the same plots. On account of the omission of sowing dates eight replicates of six treatments could then be provided. Thus the layout was a simple randomized block arrangement.

The drooping of the leaves began to be noticed by the beginning of September in all plots where the subsoil was saline irrespective of the treatments given. As the season advanced it was evident that the drooping occurred on larger areas of the field and was acuter in form in this season than it was found in 1938-39. The *tirak* had spread to parts of the field which had escaped in the previous season. The same was the case when the bolls opened. Bad opening was more pronounced and widespread in this season than in the previous one. Thus the *tirak* was more intense than what it was in the previous year. This aggravation in *tirak* as already explained in the previous contribution [Dastur and Samant, 1942] was due to a continuous spell of very dry and warm weather that prevailed in September and October.

The water deficit in the crop was accentuated by weather conditions and caused *tirak* symptoms to develop on soils which had low salinity in the subsoil.

The number of bolls per plant, the weight of seed cotton per boll, the yields and the weight of stem dry matter were recorded as before. Statistical analyses revealed the treatment variance to be of the same order as the error variance in case of boll numbers, weight of seed cotton per boll and yields. As the 'z' test indicated that the treatments were not significant it was not considered worth while to proceed further with the statistical work. The mean values for yields per plot, for the number of bolls per plant and for the weights of seed cotton per boll are given below (Table III). The general level of yields was very low because of severe *tirak*.

TABLE III

*Treatment effects on yield, boll number and boll weight*

Experiment II

	Control	Gypsum	Silt	Green manure	NP	Green manure + NP	Mean	'Z'
Yields in lb. per plot	2.01	2.92	2.35	1.71	2.55	2.71	2.38	N. S.
Number of bolls per sq. yd.	40.8	46.5	52.0	38.0	50.6	51.0	46.5	N. S.
Weight of seed cotton in gm. per boll	0.69	0.87	0.76	0.65	0.83	0.81	0.77	N. S.

No ameliorative effect of any one of the treatments was found either on opening of the bolls or the yields as was the case in the previous year.

The magnitude of differences between the yields and weights of seed cotton of the May-sown crop in the two seasons 1938-39 and 1939-40 were great as can be seen by comparison of Table III with Table II.

A  $2^4$  factorial experiment was designed in all combinations of  $\begin{pmatrix} D_1 \\ D_2 \end{pmatrix} \begin{pmatrix} S_1 \\ S_2 \end{pmatrix} \begin{pmatrix} W_1 \\ W_2 \end{pmatrix} \begin{pmatrix} O \\ N \end{pmatrix}$  where  $D_1$  = crop sown on 14 May,  $D_2$  = crop sown on 21 June,  $S_1$  = close spacing 2 ft.  $\times$  1½ ft.,  $S_2$  = wide spacing 2½ ft.  $\times$  2½ ft.,  $W_1$  = normal watering,  $W_2$  = heavy watering in September-October and  $N$  = 50 lb. of nitrogen in the form of sulphate of ammonia applied in August. The layout (shown in the Appendix) was a  $8 \times 8$  quasi-Latin square. All the four second order interactions were partially confounded with the soil differences of columns and the third order interaction was completely confounded with the soil differences of the rows. Thus an attempt was made to minimize the effect of soil heterogeneity by eliminating two-way systematic soil variations. The size of each sub-plot was 1/113 acre for sowing.

Observations on the crop under different treatments showed that the drooping of the leaves occurred from middle of September in the May-sown crop in plots with saline subsoil. This was confirmed by analyses of soil samples. The drooping symptoms were greatly reduced in such plots that received heavy waterings. No such drooping of leaves was noticed in plots which were sown in June ( $D_2$ ). The drooping of leaves was followed by shedding

by the first week of October. The bolls in such plots cracked and opened badly.

It may be recalled here that the spread and intensity of *tirak* were also greater in this field in this season of 1939-40 as compared with the previous season, i.e. 1937-38 when cotton was grown in the same field.

In a couple of plots the June-sown crop was as badly opened as the May-sown, showing no ameliorative effect of late sowings. Such plots were found to contain a greater percentage of sand than others in the first 2 ft. of the soil and small amounts of alkalinity present within 2 ft. of the surface were found to be toxic to the roots. The early and late-sown plots did not make normal growth. They were stunted in growth and produced a crop of badly opened bolls. Except for such plots or portions of such plots late-sown crop did not show *tirak*. Statistical analysis has, however, been carried out without rejecting such plots.

The number of bolls per plant and the weight of seed cotton per boll were determined from duplicate random samples. Each sampling unit comprised of six holes (two plants per hole) in  $S_1$  and three holes in  $S_2$  sub-plots. The yield, the height and the weight of stems were taken as before. The data for weight of seed per boll, the yields and the dry matter of stems

TABLE IV  
*Analyses of variances*  
(2<sup>4</sup> confounded design in 8 × 8 quasi-Latin square, 1939-40)

Due to	D. F.	Weight of <i>kapas</i> per boll		Yield of <i>kapas</i>		Dry weight of sticks	
		Mean square	F	Mean square	F	Mean square	F
Rows	7	0.1392		26.6547		822.18	
Columns	7	0.2262		28.3832		2280.62	
D	1	1.1250	8.66**	2.3831		27121.97	296.17**
W	1	0.6092	5.38*	191.1652	10.60**	285.19	3.11
N	1	0.0325		2.7183		8.34	
S	1	0.2965		191.5802	10.63**	1706.72	18.63**
D. W	1	0.5408	4.16*	165.7335	9.193**	365.29	3.90
D. N	1	0.0205		10.7339		1.79	
D. S	1	0.0914		33.1920		87.19	
W. N	1	0.0004		4.0251		21.27	
W. S	1	0.0800		5.6228		36.75	
N. S	1	0.2945		76.0166	4.22*	57.19	
D. W. N	1	0.0117		0.8374		121.60	
D. W. S	1	0.4320		56.8981		410.67	
D. N. S	1	0.0026		11.0784		24.80	
W. N. S	1	0.3927		8.2751		6.31	
Error	35	0.1299		18.0277		91.57	

\* Significant at 5 per cent level of significance

\*\* Significant at 1 per cent level of significance

were analysed by the method appropriate to the design. The details of analyses of variances are given in Table IV. The significant main effects and interactions with respective standard errors are shown in Table V under appropriate subheadings.

TABLE V  
Summary tables showing main effects and significant interactions  
Experiment III

Treatment		Average weight of kapas per boll in gm.	Difference with S. E.	Yield in maunds per acre	Difference with S. E.	Weight of stems in maunds per acre	Difference with S. E.					
Main effects	D <sub>2</sub>	1.47	** 0.18 ± 0.064	13.82	0.39 ± 1.06	22.92	** -41.17 ± 2.39					
	D <sub>1</sub>	1.29		13.43		64.09						
	W <sub>2</sub>	1.45	* 0.14 ± 0.064	15.35	** 3.46 ± 1.06	45.62	4.22 ± 2.39					
	W <sub>1</sub>	1.31		11.89		41.4						
	N	1.36		13.41	-0.17 ± 1.06	43.15	-0.72 ± 2.39					
	O	1.39	-0.03 ± 0.064	13.82		43.87						
	S <sub>1</sub>	1.43	0.10 ± 0.064	15.35	** 3.46 ± 1.06	48.67	** 10.33 ± 2.39					
	S <sub>2</sub>	1.33		11.89		38.34						
		D <sub>1</sub>	D <sub>2</sub>	Difference (± 0.091)		D <sub>1</sub>	D <sub>2</sub>	Difference (± 1.50)		D <sub>1</sub>	D <sub>2</sub>	Difference (± 3.38)
D, W Interaction	W <sub>2</sub>	1.42	1.48	0.06	W <sub>2</sub>	16.77	13.94	-2.83	W <sub>2</sub>	68.6	22.65	-45.95**
	W <sub>1</sub>	1.15	1.46	0.31**	W <sub>1</sub>	10.09	13.70	+3.61*	W <sub>1</sub>	59.6	23.2	-36.4**
	Difference	0.27**	0.02		Difference	6.68**	0.24		Difference	9.0*	55	
		± 0.091			± 1.50			± 3.38				

\* Significant at 5 per cent level of significance

\*\* Significant at 1 per cent level of significance

The three treatments which were tried for amelioration of *tirak* were: (1) deferred sowings, (2) heavy watering and (3) application of nitrogen. The last was included only as a precautionary measure though it has been already shown that deficiency of nitrogen is not the cause of bad opening on these soils. A study of Table V will show that both the ameliorative measures deferred sowings and heavy waterings, significantly increased the weight of seed cotton per boll indicating better opening of bolls, i.e. less of *tirak*. The interaction of sowing-dates with waterings (D.W) was significant showing that opening of bolls in May-sown crop was greatly improved by heavy watering while the late-sown crop showed no further improvement in opening by extra application of water, as the improvement in opening obtained by deferred sowings was of a high magnitude. The plants under late sowings did not also require extra water as no disturbance in the water balance occurred in the late-sown crop. This was indicated by absence of drooping of leaves in the late-sowing. Nitrogen, as expected, had no effect on the weight of seed cotton per boll. Thus the two remedial measures late sowing and heavy watering at the fruiting stage proved effective in improving opening of bolls, the former to a greater extent than the latter.

The generalized effect of watering on yields was significant at 1 per cent level of significance and the interaction sowing date  $\times$  watering was also significant. The early-sown crop profited considerably by extra applications of water while no benefit accrued to the late-sown. The increase in yield due to heavy watering in the former was 6.6 md. per acre while the increase was practically nil in the latter. Thus extra watering had helped the early-sown crop in increasing both the yields and the weight of cotton per boll while no similar advantage from heavy watering was derived by the late-sown in any case.

The generalized effect of spacing on yields was positive and significant indicating that the yield under close spacing was higher than that under wide spacing. Though the interaction of dates and spacing (D. S) did not come out significant, the following data (Table VIa) would show that the late-sown crop benefited more by close spacing than the early-sown. The main effect of spacing derives its significance from the significant effect of close spacing in D<sub>2</sub> only, the effect of close spacing under D<sub>1</sub> alone being non-significant.

TABLE VI

*Interaction of sowing date with spacing on yield and the effect of sowing dates on waterings on the efficiency of the plant*

(a) Average yield in maunds per acre			(b) Seed cotton per 100 gm. of stem weight (efficiency)			Mean
	D1	D2		D1	D2	
S1 . . . . .	14.44	16.27	W1 . . . . .	17.21	60.9	39.05
S2 . . . . .	12.42	11.36	W2 . . . . .	25.24	64.59	44.92
S1 — S2 . . . . .	+2.02	+4.91**	Mean . . . . .	21.23	62.74	

\*\* Significant at 1 per cent level of significance

As the early-sown plants were benefited more by watering than the late-sown plants and as wide spacing had acted against the late sowings, the generalized response to sowing dates was small and non-significant. As the interaction of sowing dates and watering was significant, no importance attaches to the generalized effects as such.

The interaction of spacing with nitrogen on yield was found to be significant. This was due to an anomalous decrease under wide spacing in the presence of nitrogen. It is possible that some of the plots with wide spacing and nitrogen came on soils which had high salinity in the subsoil.

The effects of sowing date on growth characters confirmed the conclusion already reached that late-sown plants remain stunted and produce comparatively less dry matter. Heavy waterings increased the weight of sticks significantly on early-sown only, thus bringing out a significant interaction between sowing date and watering.

The efficiency of plants for production of seed cotton was worked out in this experiment also. The effect of heavy watering and late sowing on the proportion of seed cotton produced per 100 gm. of stem dry matter is given in Table VI (b). Obviously the late sowing influences the efficiency of plant for seed cotton production. Watering is also effective but cannot compete with late sowing in this respect.

The ameliorative effects of deferred sowings on *tirak* occurring in soils with saline subsoil was further determined in another experiment arranged at the Risalewala Seed Farm, Lyallpur in the cotton season of 1940-41. The experiments discussed in the foregoing pages were conducted with 4F Punjab-American cotton variety only. It was, therefore, necessary to extend the studies by introducing in such experiments a number of *desi* and American varieties. Such a study would disclose not only the relative resistance of different American strains to *tirak*, if any, but also their suitability for adoption for late sowing.

Accordingly 18 varieties, 15 Americans and three *desis* were included in the experiment. Out of the entire lot under trial, there were six commercial varieties, four Americans and two *Desis* while the rest were newly evolved promising strains which were kindly supplied by the Cotton Research Botanist, Lyallpur.

The layout conformed to randomized blocks design with sub-plot arrangement. The entire area consisted of six blocks (320 ft.  $\times$  119 ft.) of four main plots each (80 ft.  $\times$  119 ft.). Four sowing-date treatments were distributed at random to the main plots within each block. Two rows of 119 ft. length for each of the 18 strains were accommodated in each main plot. The position of varieties within each plot was perfectly random. Non-experimental belts were cut out on all sides at pickings to avoid border effect to the main-plot treatments. There was no scope for the provision and subsequent rejection of edge rows to eliminate border effect on varietal comparisons. The marked reduction in the standard error of the varietal comparison by split plot arrangement, however, dispels the possibility of any considerable border effect influencing the sub-plot treatments. The experimental sub-plot measured 100 ft. 10 in.  $\times$  4 ft. (1/108 acre).

Sowings were done on 8 May, 23 May, 7 June and 23 June in  $D_1$ ,  $D_2$ ,  $D_3$ ,  $D_4$  plots respectively. At the time of thinning, the plants were spaced closer and closer as cotton was sown successively later. The spacings adopted for the different sowings were :  $D_1 = 2$  ft.  $\times$  2 ft.,  $D_2 = 2$  ft.  $\times$   $1\frac{1}{2}$  ft.,  $D_3 = 2$  ft.  $\times$  1 ft.,  $D_4 = 2$  ft.  $\times$  9 in. The first and the second sowings received in all eight and seven waterings respectively while each of the June-sowings was given five irrigations, i.e. three irrigations less than the first sowing. Except for such additional earlier irrigations to the May-sown plots, it was so arranged that subsequently all sowings were watered on the same date.

Yield records were maintained throughout the picking season. *Desi* varieties have to be picked every week and thus it was not convenient to record their boll weights in this experiment. Boll weight determinations from sampled plants were, therefore, confined to 15 American varieties of each sowing.

A study of analyses of variances (Table VII) revealed that there were significant variations among the four sowing dates in case of yield as well as weight of *kapas* per boll. The varietal comparisons were also highly significant on both of them. The interaction 'dates  $\times$  varieties' attained significance in yield only.

TABLE VII  
*Analyses of variances (sub-plot basis)*  
(Varietal and sowing-date experiment at Risalewala Seed Farm 1940-41)

Due to	Boll weight			Yield of <i>kapas</i>		
	D. F.	Mean square	F	D. F.	Mean square	F
Blocks . . .	5	2.3096	3.396*	5	48.5325	2.419
Dates . . .	3	12.60266	18.531**	3	116.2191	5.791**
Error (a) . .	15	0.6801	..	15	20.0695	
Main plots . .	..	..	..	23		
Varieties . .	14	1.1544	13.346**	17	140.4478	70.2239**
V $\times$ D . . .	42	0.0876		51	5.7876	2.573**
Error (b) . .	280	0.0865		340	2.2490	

<i>Components of the sum of squares due to dates of sowing</i>						
$D_1 + D_2$ vs. $D_3 + D_4$	1	27.1590	39.934**	1	135.2625	6.74*
$D_2 + D_3$ vs. $D_1 + D_4$	1	8.1421	11.972**	1	203.7615	10.153**
$D_1 + D_3$ vs. $D_2 + D_4$	1	2.5067		1	9.6332	

\* Significant at 5 per cent level of significance

\*\* Significant at 1 per cent level of significance

The total sum of squares attributable to the three degrees of freedom for the sowing dates was further split up by regarding them as quality treatments and the contributions of orthogonal components were tested against the error variance (a). The last two sowings were found to differ from the first two significantly and the differences were relatively much more pronounced on boll size than on yield. The behaviour of the central sowings was also significantly different from the two extreme sowings. It is, therefore, necessary to proceed to the detailed Table VIII to grasp the nature and magnitude of the effects of the variables under study.

There was a progressive rise in the weight of *kapas* per boll with the delay in sowing upto the third sowing beyond which there was little effect. Thus a well marked optimum towards the June sowings was clearly brought out with respect to the opening of bolls. The magnitude of increase was sufficiently high. The sowing dates stood in the order  $D_3, D_4, D_2, D_1$  according to merit and this order remained virtually the same in the different varieties taken individually. This accounted for the non-significant interaction between the two factors. Improvement in opening in all the varieties was brought about to the same extent by delay in sowing. The mean boll weights of the varieties showed significant variation among themselves. This indicates inherent varietal differences in boll sizes of the different strains. Boll weight is a composite measure of the all-round development of seed and lint of a given variety but higher boll weights in certain varieties in comparison to others, do not necessarily imply a corresponding reduction in the percentage immaturity of seeds. Varieties having large and fuzzy seeds may suffer to the same extent by *tirak* and yet may possess markedly higher seed weights due to more of non-essential parts, as compared with non-fuzzy strains under similar soil conditions.

TABLE VIII  
*Results of the experiment at Risalewala Seed Farm, 1940-41*

	AVERAGE WEIGHT OF <i>KAPAS</i> PER BOLL					MEAN YIELD IN LB. PER SUB-PLOT (1/100) ACRE				
	$D_1$	$D_2$	$D_3$	$D_4$	Mean $\pm 0.06$	$D_1$	$D_2$	$D_3$	$D_4$	Mean $\pm 0.306$
LSS . . . .	1.37	1.56	2.11	2.08	1.78	3.63	5.03	6.66	6.80	5.53
4F . . . .	1.16	1.44	1.89	1.47	1.49	2.91	4.33	7.25	4.34	4.71
289F/43 . .	1.19	1.74	2.18	1.68	1.70	2.98	4.20	5.11	4.61	4.23
289F/K25 . .	1.30	2.00	2.22	2.00	1.88	3.97	5.78	5.23	4.32	4.82
LSS early . .	1.55	1.84	2.32	2.22	1.98	4.64	9.63	11.27	9.70	8.81
289F/124 . .	1.42	1.84	2.28	2.26	1.95	5.26	6.98	7.22	6.77	6.56
289F/126 . .	1.70	2.03	2.72	2.48	2.23	5.07	6.50	8.14	7.05	6.60
289F/127 . .	1.43	1.90	2.17	2.26	1.94	4.95	6.65	4.16	4.38	5.04
289F/144 . .	1.40	1.94	2.19	2.08	1.90	4.77	5.90	6.67	5.26	5.65
289F/155 . .	0.86	1.40	1.60	1.64	1.37	2.37	4.91	4.84	4.26	4.09
289F/156 . .	1.21	1.86	2.37	2.12	1.89	4.66	7.61	8.40	6.06	6.68
289F/157 . .	1.24	1.73	2.14	1.98	1.77	5.41	6.90	6.45	5.53	6.07
289F/158 . .	1.36	1.87	2.35	2.30	1.97	4.23	7.21	7.33	6.40	6.29
289F/159 . .	1.38	2.14	2.03	2.08	1.91	5.57	8.26	6.26	5.47	6.39
289F/186 . .	1.12	1.41	1.87	1.79	1.55	3.45	4.77	6.22	5.69	5.03
DC17 . . . .						10.0	8.92	11.71	10.22	10.21
Mo1 39 . . .						12.09	10.74	15.14	13.32	12.82
Sang 119 . .						9.24	11.01	12.03	10.58	10.71
Mean . . . .	1.31	1.78	2.16	2.03		5.29	6.96	7.78	6.71	

 $\pm 0.087$ S. E. of the body of the table (interactions and varietal effects only)  $\pm 0.12$  $\pm 431$   
 $\pm 0.612$

The optimal value for yields was obtained in the third sowing after which there was a tendency for falling off in the effectiveness of further delay in sowing. This was attributable to a diminution in the boll number per unit area caused by a reduction in growth and also by some jassid attack in the last sowing in the susceptible varieties. Even then the mean yields of the fourth sowing were higher than those of the first sowing and compared favourably with those obtained from the second sowing. The varieties susceptible to jassids attained an earlier optimum in relation to sowing date than others resistant to them. This explains partly the significant interaction.

### CONCLUSIONS

The results discussed above clearly indicate that *tirak* occurring on soils which have a saline subsoil can be ameliorated by either reducing the water requirements of the crop by means of deferred sowings or by applications of extra water from the beginning of the flowering phase so that upper non-saline layers may adequately meet the demand of the crop. The first remedy of deferred sowings is to be preferred to the second as the former enables the crop to meet its own demands for water without external aid. The former is also a more practical remedy than the latter as the water supply is usually limited. There is considerable observational and experimental evidence to support the view that the late sown crop is better adapted to its edaphic and climatic environment than the early-sown (May-sown) crop. The late-sown crop shows no symptoms of water starvation and consequently is able to mature its crop of bolls under saline conditions of the soil or unfavourable conditions of weather or both. A late-sown plant is thus in physiological equilibrium with its environment and is able to stand the vagaries of weather which many a time is dry and warm during the fruiting period. It is also a more efficient organism than a May-sown plant. It produces more of seed cotton in proportion to its size than what an early-sown plant does. The latter exhausts itself in the vegetative growth and by the time bolls are formed, it has already reached a stage of senescence. A slightly higher temperature than normal for a brief spell of three weeks or so is sufficient to upset its metabolic processes on such saline subsoils, for the plant has lost its capacity for adjusting itself to such changes in its environment. No particular advantage is also gained by the sowing of the cotton crop in May even on non-saline soils as the crop exhibits a kind of photoperiodism. The flowering phase does not set in early in an early-sown and if it does, such flowers do not develop into bolls and are generally shed. The onset of flowering is not proportionately delayed as the sowings are delayed; a shift in the date of sowing does not materially influence the main flowering period which occurs in the month of September. Early sowings will be advantageous only when the flowering period is also considerably prolonged. A long flowering period would enable the crop to mature a larger number of bolls than what they do. But as the matters stand the early-sown crop becomes unbalanced with a long vegetative phase and a short reproductive phase. This lack of balance between the two phases results in a low efficiency in production of seed cotton.

The June-sown crop, however, suffers from a disadvantage as compared with the early-sown crop. As the vegetative phase is shortened the bearing points are reduced resulting in a reduced number of bolls. But this disadvantage can be counteracted by closer spacing of plants, i.e. by increasing the number of plants per acre. This measure will make up for the decrease in bearing on the late-sown plant and the crop will at the same time be less susceptible to *tirak* on saline subsoils and will produce better quality of lint.

A large number of experiments laid out on zemindars' fields have substantiated the conclusions discussed above and these results will be discussed in another contribution.

The ameliorative measures for counteracting the toxic effects of salinity in the subsoil such as the use of gypsum, silt and green manuring have not proved effective and *tirak* occurred irrespective of these treatments. These measures would also be beyond the means of zemindars, even if they were successful. There would also be difficulties of their application to the right place as *tirak* also occurs on soils which are not saline in the subsoil [Dastur and Samant, 1942].

The attempt to leach down the salts by flooding to deeper layers of soil has also not proved successful. Applications of nitrogenous fertilizers and superphosphates produced no ameliorative effect on *tirak* on such soils.

#### SUMMARY

*Tirak* or bad opening of bolls in the Punjab-American cottons on soils with saline subsoils is mainly caused by a disturbance in the water balance of the plant. A water deficit arises in the plants towards the fruiting stage which is the most critical period of plants' life and becomes more and more pronounced with the march of time. Salinity in the subsoil renders the absorption of water difficult and the plants succumb to the physiological drought. Three types of ameliorative measures were tried for counteracting toxic effect of salinity: (1) applications of gypsum, silt, farmyard manure and green manures, (2) washing down of the salts from the feeding zones of the roots by flooding of such lands and (3) efforts for preventing the development of a water deficit by means of cutting down the vegetative growth (e.g. by late sowings) or by giving extra applications of water at the fruiting stage.

Replicated field experiments were conducted to study the effects of these three types of ameliorative measures during the cotton seasons of 1938-39, 1939-40 and 1940-41 on such lands where subsoil salinity was known to exist and where *tirak* had previously occurred.

Of the three types of ameliorative measures, the two measures of the third group, viz. deferred sowings (June sowings) and extra applications of water from the flowering stage proved successful in remedying *tirak* while all the measures of the first two types failed to produce any effect.

Deferred sowings did not show drooping of leaves in October as was the case with the May-sown crop on such soils. There was also no premature defoliation. The opening of bolls (weight of seed cotton per boll) and the yields were significantly better in the June-sown crop than those of the May-sown crop. Similarly, heavy watering from fruiting stage lessened *tirak*

appreciably and increased the yields in comparison to normal waterings. Heavy watering had no effect on the opening or the yield of June-sown crop as the latter did not stand in need of extra water and was not profited by it. Late sowing was found to be superior to heavy watering in effect on *tirak*.

June sowings produced less number of bolls per plant than May sowings on account of a reduction in the vegetative growth in the former. This was a disadvantage in late sowings but it was successfully counteracted by increasing the number of plants per acre. This could be done by adopting closer spacing of plants.

The experiment laid out in 1940-41 was a varietal-cum-sowing-date trial. There were four sowing dates equally spaced at fortnightly intervals commencing from the second week of May with 15 American varieties and three *desi* varieties.

The results of boll size (weight of seed cotton per boll) showed that opening of bolls improved as the sowings were delayed. The opening of bolls in the two June sowings was significantly better than that of the May sowings. The improvement in opening was universal to all the varieties included for study. Similarly, the mean yields of the former were significantly higher than those of the latter. Varieties differed in their adaptability to late sowing. The strains resistant to jassids were in general better suited to the June sowing while those susceptible to them had a well marked optimum towards the central sowings (end of May to second week of June). The first sowing gave lower yields under all varieties taken individually.

The crop when sown in June is in a physiological equilibrium with its environment on soils with saline subsoil. It does not suffer from water starvation on such soils while the May-sown crop does. The production of seed cotton in proportion to plant size (dry weight) is much higher in late-sown as compared with that of the early-sown. The disadvantage of reduction in bearing which usually accompanies this practice can be counteracted by increasing the number of plants per acre by reducing the distance between the rows and adopting closer spacing from plant to plant within the rows.

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## APPENDIX

*Layout plan with treatments and yields in maunds per acre of the 2<sup>4</sup> factorial design on 4-F cotton in sq. 27 D<sub>1</sub>, 1939-40  
(8 × 8 quasi-Latin square)*

Column No. 1	D1 W1 S1 8.15	D1 W1 S2 9.38	D2 NW1 S2 13.34	D2 NW1 S1 25.39	D1 NW2 S1 12.81	D1 NW2 S2 20.58	D2 W2 S2 15.19	D2 W2 S1 21.01
Column No. 2	D1 NW2 S1 21.63	D2 W1 S1 17.22	D2 NW2 S1 17.72	D1 W1 S1 15.04	D1 NW1 S2 7.57	D2 W2 S2 10.71	D2 NW1 S2 14.88	D1 W2 S2 21.22
Column No. 3	D1 W2 S2 20.99	D2 NW1 S2 10.73	D1 W1 S2 12.69	D2 NW2 S2 10.16	D2 W2 S1 15.23	D1 NW1 S1 9.57	D2 W1 S1 14.78	D1 NW2 S1 17.66
Column No. 4	D1 NW1 S2 8.52	D1 NW2 S2 13.62	D2 W2 S2 10.25	D2 W1 S2 10.98	D1 W1 S1 22.66	D2 NW2 S1 12.79	D1 W2 S1 12.24	D2 NW1 S1 18.54
Column No. 5	D2 W1 S2 11.59	D1 NW1 S1 3.23	D2 W1 S1 10.03	D1 NW1 S2 6.77	D1 W2 S2 17.70	D1 W2 S1 10.78	D2 NW2 S1 18.14	D2 NW2 S2 9.86
Column No. 6	D2 W2 S1 16.34	D2 NW2 S1 13.89	D1 NW2 S2 6.30	D1 W2 S2 20.14	D2 W1 S2 11.44	D2 NW1 S2 5.01	D1 NW1 S1 6.01	D1 W1 S1 7.21
Column No. 7	D2 NW2 S2 10.24	D2 W2 S2 11.18	D1 W2 S1 14.99	D1 NW2 S1 28.37	D2 NW1 S1 16.45	D2 W1 S1 10.89	D1 W1 S2 8.08	D1 NW1 S2 7.45
Column No. 8	D2 NW1 S1 14.40	D1 W2 S1 17.50	D1 NW1 S1 23.19	D2 W2 S1 17.46	D2 NW2 S2 12.79	D1 W1 S2 5.96	D1 NW2 S2 11.75	D2 W1 S2 13.52
Row No.	1	2	3	4	5	6	7	8

# RECOVERY OF WHITE SUGAR FROM THE PUNJAB AND THE UNITED PROVINCES CANES

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THE investigation described in this paper was carried out during the two cane seasons 1937-38 and 1938-1939, in order to throw light on the alleged differences in the percentage recovery of sugar from canes imported into the Punjab from the United Provinces for the then existing Sonapat Sugar Factory and the corresponding canes grown locally. The Punjab Sugar Corporation reported that the recovery for local Sonapat canes was 7.7 per cent as against 10.18 per cent for corresponding canes imported from Amroha and elsewhere in the United Provinces. A systematic analytical survey of the canes grown in the two provinces, combined with investigations of the soils on which the canes were actually grown, was accordingly carried out in order to throw light on the differences in percentage recovery recorded. The canes in the particular areas in the Punjab and the United Provinces in which this investigation was carried out in 1937-38 unfortunately experienced an exceptionally bad attack of pyrilla, which to a considerable degree rendered the results less conclusive than was desired. Valuable data were, however, collected during the first year's survey but owing to the abnormal conditions it was repeated in the following year, i.e. 1938-39, as it is obvious that in order to obtain reliable comparative data on such a matter as this the data must be collected during a season when normal conditions prevail. It is, however, almost impossible to find any extensive area during any season, where what might be called 'absolutely normal conditions' do prevail. For example during the second year of the investigation although pyrilla was practically absent, the canes in both the provinces were subjected to drought of greater intensity than during the first year.

The United Provinces grow more than half the total cane produced in India, the chief tracts being the Meerut, the Rohilkhand division in the west, and the Gorakhpur division in the extreme east.

For the investigation under report eight localities were selected, four from the western United Provinces, namely Meerut, Bisokhar, Salimpur and Amroha, and four from the south-eastern Punjab, namely Karnal, Tharu, Rohat and Jaukhli. In the 1938-39 survey, the districts in the Punjab were the same but in the United Provinces, Billari village in the Moradabad district and Shah-jahanpur were added and Bisokhar omitted.

Owing to the above-mentioned attack of pyrilla and the removal of the Sonapat Sugar Factory to Amroha there were only 2,895 acres under

sugarcane in the 1938-39 season in the Sonapat tehsil, as against 11,557 in the 1937-38 season. Also, very little of this cane of the second year was used for the manufacture of *gur* or sugar but was chiefly employed as fodder on account of famine. Similar conditions prevailed in the United Provinces, where a number of factories did not work for more than two months.

### CLIMATE

The western part of the United Provinces receives a higher rainfall than the south-eastern Punjab, the monsoon usually setting in about the middle of June from which date monsoon conditions prevail until about the end of September. Furthermore, the eastern portion of the area in the U. P. under report usually gets a considerably higher rainfall than the western parts as shown from the data in Table I. The rainfall, however, during the monsoon prior to the first year's investigation was below normal in all the localities investigated and this factor occurring at a period when the cane is at a stage of maximum growth caused the crop to be stunted both in the United Provinces and the Punjab. This undoubtedly constituted one of the predisposing causes to the severe attack of pyrilla during the ensuing cane season, during which there were occasional frosts which, however, were not severe enough to do any harm. The major trouble was pyrilla.

TABLE I

*Rainfall in inches during the last five years at the localities under survey*

Locality	Years					Average
	1934	1935	1936	1937	1938	
Meerut (Western United Provinces)	30.25	21.52	23.46	17.88	9.79	20.6
Muzaffarnagar (for Salimpur) (Eastern United Provinces.)	36.24	31.43	41.58	32.51	26.32	33.6
Amroha (Eastern United Provinces)	22.99	39.39	50.03	26.99	31.95	34.5
Shahjahanpur (Eastern United Provinces)	38.04	25.75	70.23	22.78	39.43	41.2
Sonapat (South-eastern Punjab)	17.35	17.25	23.85	19.92	9.32	17.5
Karnal (South-eastern Punjab.)	31.74	43.15	34.26	25.09	18.60	30.5

We have already mentioned how the trouble from pyrilla was reflected to a certain extent in a smaller acreage under cane in 1938-39 than in 1937-38 and how difficult it is to find, what may be called, a normal season. In the first year the rainfall in the previous monsoon was below normal and was combined with an attack of pyrilla and in the second year although there was freedom from pyrilla yet the rainfall in the previous monsoon was lower than in the first year both in the Punjab and the United Provinces, particularly at Meerut and Sonapat. As a result of this deficient rainfall during the period when the

cane crop most needed it, the crop in both the provinces was stunted and consequently matured earlier. In the 1938-39 cane season, there was no frost and consequently quality was maintained throughout the short crushing season.

#### SOILS AND GENERAL AGRICULTURAL PRACTICES

The nature of the soils from that part of the United Provinces investigated and of the south-eastern Punjab varies from sandy to medium loams tending to somewhat heavier types in the subsoil. On the whole, however, the analytical data from these soils and agricultural experience show them to be eminently suited, in both provinces, to the production of cane. Average analytical data computed from separate investigations in a number of localities in both the provinces are given below :—

#### *Percentage on air-dried soils*

Depth	Total nitro- gen	Organic matter	Ex- change- able calcium	Avail- able $P_2O_5$	Maxi- mum water- holding capacity	Water- soluble salts	Clay	pH
United Pro- vinces—								
1st foot .	0·0553	0·650	0·096	0·055	37·3	0·117	13·4	6·58
2nd foot .	0·0448	0·426	0·144	0·042	37·0	0·102	21·2	6·72
3rd foot .	0·0392	0·376	0·187	0·032	38·5	0·090	24·8	6·81
Punjab—								
1st foot .	0·0740	0·900	0·158	0·035	40·7	0·200	19·4	6·95
2nd foot .	0·0548	0·706	0·182	0·030	40·6	0·170	25·7	6·78
3rd foot .	0·0496	0·583	0·186	0·031	40·6	0·178	27·6	6·62

An interesting point brought out in the survey was that the soils of the south-eastern Punjab, are comparatively heavier and contain a greater amount of exchangeable calcium than the soils from the western United Provinces. There is not much difference in the reaction of the two soils although the United Provinces soils have slightly lower pH values. The differences in the value of the two sets of soils for sugarcane production appear to lie in the differences in the amounts of water-soluble salts and exchangeable calcium in the soils of the two provinces. In the Punjab the soils contain a considerable concentration of water-soluble salts which accumulate near the surface, but in the United Provinces, where rainfall and humidity are both greater and where the soil is lighter, the percentage of water-soluble salts is much less. It is perhaps this fact more than any other which may influence the ash content of the sugarcane juice and subsequent percentage recovery of sugar in the factory, and which may consequently form an explanation of the alleged difference in the value of two sets of canes in sugar production. As shown by Lander and

Ramji Narain [1936], it is the greater amount of ash in the juice from the Punjab canes rather than its slightly lower sucrose content as compared with the juice of the sugarcane from the United Provinces, which is responsible for the lower net rendiment value of the *gurs* made from the Punjab canes. There are certain other factors also which appear to have a definite bearing both on the quantitative and qualitative production of cane in the United Provinces and the Punjab. The most important of these appear to be climatic in regard to the relative degree of rainfall, and the variations in certain important aspects of agricultural procedure. In the United Provinces *barani* land usually produces a moderately good crop of cane which could not be produced on corresponding lands in the Punjab. Again, average well-cultivated soils in the Punjab usually receive far more natural manure than the average corresponding United Provinces soils, which accounts for the fact that the former are generally richer in nitrogen and organic matter than the latter. There are exceptions, however, and in some parts of the United Provinces, intensive manuring produces soils richer in nitrogen and organic matter than is found in average Punjab soils. Again, the cost of cultivation owing to general economic conditions is lower in the United Provinces than in the Punjab, for which reason cane is a more extensively grown crop in the former province. Of the total cultivated area in the localities surveyed, about 20 per cent is under cane in the United Provinces, against 10 per cent in the Punjab. On the other hand, the standard of cultivation and the amount of manure used are, as a rule, higher in the Punjab, so that land irrigated by well or canal water in the Punjab generally produces more cane per acre than corresponding land in the United Provinces.

#### PESTS

As already mentioned, the 1937-38 cane crop in both the western United Provinces and south-eastern Punjab was severely damaged by pyrilla. The high-yielding varieties, such as Co 312, suffered most, and the more luxuriant and succulent the leaves the heavier was the attack. The natural result was a juice of inferior quality with a diminished sucrose content and so high a glucose content that *gur* could not be prepared from it. As a typical example the following table shows the deterioration of Co 312 as a result of this attack.

*Co 312 (Karnal) attacked by pyrilla*

Date of analysis	Percentage on sugar cane						Purity coefficient
	Juice	Sucrose	Glucose	Total sugars	Total solids	Glucose ratio	
9 Dec. 1937	71.8	3.2	1.65	4.9	6.1	51.1	52.9
27 Dec. 1937	75.6	4.3	2.08	6.4	7.8	48.4	53.5
12 Jan. 1938	74.9	4.0	1.81	5.8	7.3	45.2	57.7
24 Jan. 1938	74.6	3.7	1.82	5.5	6.6	49.2	56.2
8 Feb. 1938	75.2	4.8	1.79	6.6	7.6	37.3	63.4

During the following season the crop was free from pyrrilla and the cane ripened normally as shown below :—

*Co 312 (Karnal) free from pyrrilla (1938-39)*

Date of analysis	Juice	Sucrose	Glucose	Total sugars	Total solids	Glucose ratio	Purity coefficient
9 Dec. 1938	69.3	9.6	0.73	10.3	11.6	7.6	82.8
19 Dec. 1938	67.9	8.9	0.29	9.2	10.7	3.0	83.2
13 Jan. 1939	67.6	11.2	0.26	11.5	12.6	0.9	88.9
29 Jan. 1939	71.6	11.3	0.10	11.4	12.8	1.2	88.3
14 Feb. 1939	66.7	10.1	0.14	10.2	12.3	1.4	82.1
23 Feb. 1939	72.1	11.0	0.14	11.1	12.5	0.8	88.0

PLAN OF WORK DURING THE SURVEYS

In considering the data from different localities surveyed it may be mentioned that all the varieties at each place were analysed six times during each season from the beginning of December till the first week of March. It is not proposed to give all the analytical data collected each year, but only the average composition of different varieties, together with figures for the glucose ratio, purity coefficient and saline coefficient for the maturity periods. The figures for the yield of stripped cane, total solids and sucrose per acre, have also been included. The soils from the fields from which the canes were analysed were sampled at a number of places to a depth of 3 ft. and corresponding 1 ft. samples from all the bores were mixed together, so that three composite samples were obtained from each locality for analysis.

LOCALITIES SURVEYED

*The United Provinces*

*Government Agricultural Farm, Meerut*

The soil of this farm is an average loam but becomes somewhat heavier below the first foot. The average composition of the soils of this farm is shown below :—

*Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	Water-soluble salts	pH	Exchangeable calcium	Available $P_2O_5$	Clay	Silt	Sand
1st foot	0.0680	0.970	0.180	7.81	0.120	0.046	15.1	25.4	59.44
2nd foot	0.0476	0.421	0.160	8.34	0.136	0.030	24.9	31.4	43.70
3rd foot	0.0476	0.405	0.180	8.54	0.124	..	27.7	32.8	39.48

The soil is alkaline in reaction, possesses a moderate amount of exchangeable calcium and a moderately high percentage of water-soluble salts.

Four varieties of canes were examined, viz. Co 244, Co 312, Co 313 and Co 331, the analytical data from which are given in Table II.

TABLE II  
*Analytical data of the varieties of cane grown at the Government Agricultural Farm, Meerut*

(Average during the ripening period)

Par als	Percentage on cane							Glu- cose ratio	Purity Saline coeffi- cient	Yield per acre in maunds			Ripening period		
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash			Cane	Sucro- se	Total solids			
Co 244	1937-38	71.7	9.7	0.59	10.5	11.4	0.9	0.409	6.1	83.1	24.0	586.3	56.8	66.8	5/1 to 14/2
	1938-39	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Co 312	1937-38	72.3	8.8	0.80	9.6	10.8	1.2	0.405	9.1	81.4	22.0	920.6	81.0	99.4	31/1 to 14/2
	1938-39	69.2	9.6	0.37	10.0	11.3	1.3	0.399	4.1	85.3	25.7	940.0	90.2	106.2	18/12 to 20/2
Co 313	1937-38	68.1	10.1	0.25	10.4	11.4	1.0	0.548	2.4	88.6	18.5	547.9	55.3	62.4	17/1 to 14/2
	1938-39	..	10.7	0.18	10.9	12.3	1.4	0.482	2.0	86.2	24.1	659.9	70.6	81.2	6/12 to 20/2
Co 331	1937-38	69.3	9.0	0.50	9.5	10.8	1.3	0.503	5.5	85.3	18.0	607.1	54.6	65.5	31/1 to 14/2
	1938-39	67.7	9.8	0.45	10.3	11.4	1.1	0.451	3.6	85.8	22.7	865.0	84.7	98.6	18/12 to 20/2
Average	1937-38	70.4	9.4	0.56	10.0	11.1	1.1	0.466	5.8	85.6	21.1	666.0	61.9	73.5	..
	1938-39	68.0	10.0	0.29	10.3	11.6	1.3	0.455	3.2	85.8	23.8	821.7	81.8	95.3	..

Co 312 gave the highest outturn of 920·64 maunds per acre in 1937-38, and 940 maunds in the following season, but its maturity was somewhat delayed owing to the heavy manuring practised at the farm, and a larger number of irrigations than is usually given in the United Provinces.

The most important factors to be kept in mind in connection with the specific object of this survey are the ash content of the juice and the saline coefficient, the latter being the ratio between sucrose and ash. The canes from this farm showed the highest ash content and the lowest saline coefficient of any examined in the United Provinces, and were accordingly poorer in quality. It may be noted that in the 1937-38 season, yields from all varieties were lower than in 1938-39, probably owing to the attack of pyrilla in the former season, which was more severe at this place than at any other surveyed in the United Provinces.

### *Bisokhar*

Bisokhar is one of the villages situated in the new sugar development centre of the United Provinces and its soils are good sandy loams as their average composition given below shows :—

#### *Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available $P_2O_5$	Water-soluble salts	Clay	Silt	Sand
1st foot	0·0580	0·840	6·67	0·118	0·032	0·080	13·6	16·8	69·6
2nd foot	0·0420	0·474	7·22	0·134	0·028	0·100	19·1	16·5	64·4
3rd foot	0·0336	0·484	6·86	0·154	0·028	0·160	22·9	18·9	58·2

These soils contain a smaller percentage of water-soluble salts than those of the Meerut farm, whilst the organic matter and total nitrogen content is much the same.

Four chief varieties were examined at Bisokhar, viz. Co 244, Co 312, Co 313 and Co 331 (Table III). The last two had received a dressing of a mixture of ammonium sulphate and castor cake at the rate of five maunds per acre, which may have been responsible for the high ash content of the juice. It is interesting to note that the ash content of Co 244 and Co 312, which were unmanured here, was much less than that of the corresponding manured cane at the Meerut farm. This suggests that manuring may be one of the causes responsible for an increase in the amount of mineral matter in the juice of cane. The purity coefficient figures of the manured Co 313 and the unmanured Co 244, both of which are early-ripening varieties, indicate that manuring delays ripening—an observation which is also borne out by a consideration of the analytical data for Co 313 at Amroha which received no manures.

TABLE III  
*Analytical data of the varieties of cane grown at village Bisokhar (1937-38)*

Particulars	Percentage on cane						Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period	
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars				Ash	Cane	Sucro- se		Total solids
Co 244 .	68.1	11.1	0.53	11.6	12.4	0.8	0.302	4.9	89.5	39.4	350.0	38.4	43.4	3/12 to 16/2
Co 312 .	71.0	9.2	0.78	10.0	11.1	1.1	0.237	8.5	85.3	38.9	550.5	50.7	55.1	16/2
Co 313 .	70.3	10.3	0.36	10.7	12.1	1.4	0.647	3.5	85.1	16.3	640.5	66.0	77.5	17/12 to 16/2
Co 331 .	70.4	9.1	0.66	9.8	11.9	1.1	0.497	6.1	85.0	19.1	650.0	59.1	72.1	19/1 to 16/2
Average .	70.0	9.9	0.58	10.5	11.9	1.4	0.420	6.1	85.0	23.4	547.8	53.7	62.0	..

*Salimpur*

This village situated about three miles from Muzaffarnagar has light loam soils similar to those of the Meerut farm, but they tend to become heavier below the first foot. The average composition of these soils is given below :—

*Percentage on air-dried soil*

Depth-	Total nitro- gen	Organic matter	pH	Ex- change- able calcium	Avail- able P <sub>2</sub> O <sub>5</sub>	Water- soluble salts	Clay	Silt	Sand
1st foot	0·0588	0·710	6·25	0·120	0·030	0·160	15·7	25·9	58·3
2nd foot	0·0532	0·307	6·25	0·166	0·046	0·160	25·7	28·2	46·1
3rd foot	0·0504	0·226	6·25	0·200	0·025	0·080	30·7	28·3	40·7

This soil is heavier than those of Bisokhar and Amroha and is adequately rich in nitrogen, organic matter and exchangeable calcium, and a uniform pH value of 6·25 down to 3 ft. in depth was found, indicating its acidic nature.

The four varieties examined here, viz. Co 213, Co 244, Co 312 and Co 313 (Table IV) were all ripe at the end of November or in the first week of December and their low ash content and high saline coefficients show them to be superior to the canes from Meerut and Bisokhar. The outturn, however, was poorer. Co 312, for example, a heavy yielding cane gave only 425 maunds of cane per acre. Compared with Amroha canes all varieties showed a low saline coefficient but gave higher yields.

TABLE IV  
*Analytical data for the cane varieties grown at village Salimpur*

Particulars	Percentage on cane							Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				Cane	Sucro- se	Total solids	
Co 244 : 1937-38	69.9	11.4	0.58	12.0	13.0	1.1	0.349	5.0	87.2	33.7	375.0	42.7	48.7	8/12 to 20/2
1938-39	64.7	10.9	0.22	11.1	12.3	1.2	0.286	2.7	87.8	38.0	400.0	45.6	49.2	4/12 to 28/2
Co 213 : 1937-38	67.5	10.7	0.39	11.1	12.3	1.2	0.478	3.7	87.0	29.9	350.0	37.4	43.0	8/12 to 20/2
1938-39	65.7	10.3	0.48	10.8	12.3	1.5	0.408	5.0	83.6	25.1	370.0	38.1	45.5	4/12 to 28/2
Co 313 : 1937-38	70.0	11.4	0.39	11.8	12.8	1.0	0.421	3.4	89.1	27.9	410.0	46.1	52.5	8/12 to 20/2
1938-39	64.0	11.3	0.29	11.6	12.8	1.2	0.385	2.7	88.7	29.5	400.0	45.2	51.2	4/12 to 26/1
Co 312 : 1937-38	70.8	9.7	0.90	10.6	11.7	1.1	0.340	9.3	82.9	29.5	425.0	41.2	49.7	8/12 to 20/2
Average: 1937-38	69.6	10.8	0.57	11.4	12.5	1.1	0.397	5.4	86.6	28.4	390.0	41.9	48.5	..
1938-39	64.8	10.8	0.33	11.1	12.4	1.3	0.359	3.4	86.6	30.9	390.0	42.3	48.6	..

*Amroha*

This village is situated in the Rohilkhand division and lies near the hills, and for this reason the rainfall is considerable, and the climate more humid than that of Meerut and Bisokhar or the south-eastern districts of the Punjab. As may be seen from the table below, the soil is a sandy loam and, as judged from its content of organic matter, exchangeable calcium, water-soluble salts and total nitrogen, is the poorest of all the soils of the four localities investigated in the United Provinces. It is interesting to record that although the annual rainfall at Salimpur and Amroha is practically the same as each place, and other climatic aspects are similar, yet the Amroha soils contain the least amount of exchangeable calcium of any of those examined during this survey either in the United Provinces or the Punjab. The Amroha soils are very light and this may partially account for their low content of exchangeable calcium—an observation which appears to hold good also for the soils of Jaukhli in the Punjab. The average composition of the Amroha soils is as follows :—

*Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available $P_2O_5$	Water-soluble salts	Clay	Silt	Sand
1st foot	0·0392	0·484	6·40	0·060	0·044	0·070	12·2	15·6	68·1
2nd foot	0·0384	0·484	6·18	0·098	0·035	0·050	19·7	18·2	62·3
3rd foot	0·0280	0·484	5·92	0·100	0·021	0·060	22·2	18·1	59·7

All the four varieties examined at Amroha (Table V), viz. Co 213, Co 312, Co 313 and Co 331 matured early and had a low ash content but a high purity coefficient, sucrose content and saline coefficients. Thus, the quality of the cane was the best of any in the United Provinces or the Punjab. For example, Co 331 had a sucrose content of 10·1 per cent, whilst the other three varieties had more than 11 per cent. It may be noted, however, that whereas the quality of cane produced was excellent, the outturn per acre was the lowest found. Co 213, which gave high yields at other places, gave only 256 maunds of cane per acre at Amroha. From the factory point of view, quality is more important than quantity, but the cultivator's chief concern is quantity and in this respect interests conflict.

TABLE V  
*Analytical data of cane varieties grown at Amroha*

Particulars	Percentage on cane							Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				Cane	Sucro- se	Total solids	
Co 213 : 1937-38	68.1	11.2	0.35	11.6	12.6	1.0	0.364	3.1	88.9	33.0	256.0	28.7	32.2	12/2 to 22/2
1938-39	63.9	11.4	0.35	11.8	13.0	1.2	0.318	3.6	88.3	36.6	220.0	25.1	28.6	11/12 to 19/2
Co 312 : 1937-38	71.7	11.2	0.54	11.7	12.6	0.9	0.219	4.8	88.9	60.1	350.0	39.2	44.1	6/1 to 22/2
1938-39	68.5	11.5	0.51	12.0	12.9	0.9	0.188	4.7	89.2	62.2	300.0	34.5	36.0	30/11 to 19/2
Co 313 : 1937-38	66.6	11.9	0.34	12.3	13.1	0.8	0.243	2.9	90.8	52.3	300.0	35.7	39.3	12/12 to 22/2
1938-39	65.6	12.3	0.25	12.6	13.8	1.2	0.246	2.2	89.6	52.3	270.0	33.2	37.2	30/10 to 12/2
Co 331 : 1937-38	69.0	10.1	0.78	10.9	11.8	0.9	0.213	7.7	85.6	49.0	341.7	34.5	40.3	12/12 to 22/2
1938-39	66.0	12.0	0.32	12.3	13.5	1.2	0.188	2.8	90.0	70.0	290.0	34.0	39.1	30/11 to 19/2
Average: 1937-38	68.9	11.1	0.50	11.6	12.5	0.9	0.260	5.1	88.6	42.7	312.0	34.5	39.0	..
1938-39	66.0	11.8	0.33	12.1	13.3	1.1	0.225	3.5	89.3	55.3	270.0	31.9	35.2	..

In this connection the following comparative figures for Meerut and Amroha are of interest :—

*Maunds per acre*

Locality	Season	Yield	Sucrose	Glucose	Ash	Available cane-sugar
Meerut	1937-38	666·0	62·0	3·84	3·10	49·6
	1938-39	822·0	81·8	2·46	3·74	66·2
Average	..	744·0	71·9	3·15	3·42	57·9
Amroha	1937-38	312·0	34·5	1·56	0·82	31·2
	1938-39	270·0	31·9	0·81	0·62	28·9
Average	..	291·0	33·2	1·19	0·72	30·1

It will be seen that, considering both the seasons, the amount of white sugar which could be obtained from an acre of sugarcane at Meerut was almost double that from the same area at Amroha, and from the point of view of the cultivator attempts to increase the yield deserve encouragement, but there appears to be a limiting point beyond which an increase in tonnage reflects in a decrease in quality. The interests of the factory and the cultivator cannot be identical so long as the price of cane is fixed irrespective of quality. Another point noted in connection with the cane crop at Amroha was that the canes ripened from five to seven weeks earlier than those at Meerut. All factors which tend to increase the vegetative growth must delay ripening, and of these the amount of irrigation and manure applied are the most important. However, we find that the total amount of water, i.e. rain plus irrigation, applied to the cane crop at Amroha and at Meerut, was the same. As we have seen, the Meerut canes investigated received, in the 1937-38 season, five maunds of a mixture of castor cake and ammonium sulphate, and it is problematical whether this amount of manure could have delayed the ripening of cane by as long as five weeks. It is to be noted, however, that in the season 1938-39 the canes at Bilari were heavily manured, for in addition to the basic dressing of green manure with 300-400 maunds of sunn-hemp they received also two maunds of ammonium sulphate and 15 maunds of castor-cake per acre. These canes ripened about the end of January, whereas the Meerut canes which had received less manure than the above ripened about the middle of December, and the Amroha canes which were raised without any manure were ripe as early as the end of November. It would seem, therefore, that manuring does delay ripening, and in proportion to the amount of manure applied. Another characteristic of the crop at Amroha was the fact that during both the seasons the ash content of the cane, after it had reached maturity, progressively decreased as long as the cane remained standing in the field.

It will thus be seen that the juice from canes at this station showed a low ash content and high purity and saline coefficients. Although the soil was not particularly good yet Amroha produced the best sugarcane found in the United Provinces and the Punjab. Co 313 in 1938-39 had a sucrose content of 12·3 and the others only slightly less. Although the cane produced was of very high quality yet the outturn per acre was low and thus suited the needs of the factories, but was not so satisfactory from the cultivator's point of view, where quantity matters most.

*Bilari (1938-39 only)*

The soil of this village in the Moradabad district is a medium loam, rich in nitrogen, organic matter and exchangeable calcium and has an acidic reaction. For average composition see below :—

*Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available $P_2O_5$	Water-soluble salts	Clay	Silt	Sand
1st foot	0·056	0·684	6·19	0·106	0·0740	0·100	15·8	22·0	62·20
2nd foot	0·0504	0·555	6·42	0·186	0·0468	0·060	30·0	20·0	50·0
3rd foot	0·0420	0·439	6·67	0·178	0·0521	0·060	35·2	..	..

Four varieties of cane were examined, namely Co 213, Co 312, Co 313 and Co 331 (Table VI). All were green manured with sunn-hemp with the addition of two maunds of ammonium sulphate and 15 maunds of castor-cake per acre with the result that the outturn of cane was heavy compared with other places in the United Provinces. This heavy manuring not only delayed maturity but also decreased the sucrose content and the purity coefficient and increased the ash compared with the unmanured Amroha canes. The recovery of sugar by the open-pan system as reported by the Sugar Experiment and Testing Station at Bilari was only 5·5.

TABLE VI  
*Analytical data for cane varieties grown at village Bilari (1938-39)*

Particulars	Percentage on cane						Yield per acre in maunds			Ripening period				
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash	Glu- cose ratio	Purity coeffi- cient		Saline coeffi- cient	Cane	Sucro- se	Total solids
Co 213	71.4	9.6	0.89	10.4	11.9	1.5	0.396	8.4	80.5	25.4	600.0	57.6	71.4	15/1 to 22/2
Co 312	70.0	11.0	0.63	11.6	12.7	1.1	0.255	6.4	86.5	43.0	850.0	93.5	108.1	20/2 to 22/2
Co 313	71.3	10.7	0.85	11.6	12.9	1.3	0.282	9.9	82.6	44.7	800.0	85.6	103.2	8/12 to 22/2
Co 331	68.3	9.9	0.59	10.5	11.4	0.9	0.270	4.1	86.4	37.6	1100.0	108.8	125.4	15/1 to 22/2
Average	70.2	10.3	0.71	11.0	12.2	1.2	0.281	7.2	83.9	47.7	837.5	86.3	100.2	..

TABLE VII  
*Analytical data for cane varieties, grown at Shahjahanpur (1938-39)*

Particulars	Percentage on cane					Glu- cose ratio	Purity coeffi- cient	Yield per acre in maunds			Ripening period			
	Juice		Non- sugars		Cane			Total solids						
	Sucro- se	Glu- cose	Total sugars	Total solids										
Co 213	68.6	9.5	0.59	10.1	11.5	1.4	0.271	9.8	83.5	35.9	600.0	57.0	67.8	2/12 to 27/2
Co 312	66.1	10.4	0.51	10.9	12.0	1.1	0.247	5.9	87.0	42.1	800.0	83.2	96.0	2/12 to 27/2
Co 313	68.4	11.8	0.24	12.0	13.1	1.1	0.304	3.2	89.6	38.8	700.0	88.5	98.3	2/12 to 27/2
Co 331	66.4	10.1	0.45	10.6	11.6	1.0	0.238	6.4	86.2	47.6	800.0	80.8	92.8	2/12 to 27/2
Average	67.6	10.4	0.42	10.8	12.0	1.2	0.266	6.3	86.6	41.1	737.5	77.3	88.3	..

*Shahjahanpur (1938-39 only)*

The Shahjahanpur district is excellently suited for sugarcane having an annual rainfall of about 40 in. and a good acid sandy loam soil, low in water-soluble salt content. For average composition see table below :—

*Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available $P_2O_5$	Water-soluble salts	Clay	Silt	Sand
1st foot	0.0448	0.400	6.24	0.072	0.0637	0.076	8.0	8.40	83.6
2nd foot	0.0364	0.362	6.40	0.132	0.0498	0.080	16.2	34.80	49.0
3rd foot	0.0280	0.324	6.67	0.132	0.0310	0.080	18.0	14.20	67.80

Four varieties of cane were examined, viz. Co 213, Co 312, Co 313 and Co 331 (Table VII) which were green manured with sunn-hemp plus two maunds of castor-cake and one maund of ammonium sulphate. The outturn of cane, which was of good quality, was greater than that from Amroha and Salimpur but inferior in quality to the Amroha cane. The yield was from 600 to 800 maunds per acre and all varieties matured early in December.

*The Punjab*

The Karnal and Rohtak districts are regarded as the best sugarcane-growing tracts in the Punjab, the climate being more suitable for cane than anywhere else in the province, with the result that in normal years high yields are obtained. Four localities were selected for the investigation, viz. Tharu, Jaukhli, Rohat (near Sonapat) and Karnal.

*Tharu*

It will be seen from the table below that the soil of this village is a clay loam, rich in organic matter, total nitrogen, exchangeable calcium and water-soluble salts. The soil is acidic in reaction and becomes more so with increasing depth. It has the following average compositions :—

*Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available $P_2O_5$	Water-soluble salts	Clay	Silt	Sand
1st foot	0.0840	1.035	6.75	0.176	0.081	0.200	32.2	23.3	53.5
2nd foot	0.0588	0.484	6.30	0.248	0.042	0.156	30.5	25.4	44.1
3rd foot	0.0532	0.469	6.30	0.232	0.042	0.140	32.8	22.7	44.5

TABLE VIII  
*Analytical data for cane varieties grown at village Tharu*

Particulars	Percentage on cane							Glu- cose ratio	Purity coeff- cient	Saline coeff- cient	Yield per acre in maunds			Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				Cane	Sucro- se	Total solids	
Co 244 : 1937-38	71.0	10.3	0.55	10.9	12.0	1.1	0.370	5.3	85.8	27.5	750.0	77.2	90.0	15/2
1938-39	65.8	8.9	0.75	9.7	11.0	1.3	0.341	8.9	81.0	25.1	800.0	71.2	88.0	..
Co 312 : 1937-38	73.2	6.1	1.30	7.4	8.4	1.0	0.687	21.1	72.2	10.1	850.0	51.8	71.4	16/1
1938-39	64.3	9.9	0.48	10.4	11.8	1.4	0.331	4.8	83.0	30.0	850.0	84.10	100.3	..
Co 313 : 1937-38	72.0	9.4	0.62	10.0	11.2	1.2	0.599	6.6	83.9	15.6	810.7	76.1	90.7	15/2
1938-39	64.9	10.1	0.41	10.5	12.0	1.5	0.452	3.8	83.8	23.8	800.0	80.0	96.0	..
Co 331 : 1938-39	63.0	8.6	0.79	9.4	10.9	1.5	0.439	9.0	78.9	20.2	875.0	75.2	93.4	..
Average : 1937-38	72.1	8.6	0.82	9.4	10.5	1.1	0.545	11.1	80.6	17.7	803.6	68.3	84.0	..
1938-39	64.4	9.4	0.61	10.0	11.4	1.4	0.391	6.6	81.7	24.7	831.0	77.8	87.97	..

As already noted the crop suffered particularly severely at this place from *pyrilla* in 1937-38, hence the analytical data regarding the composition of the cane cannot be regarded as normal. Of the three varieties, viz. Co 244, Co 312, Co 313 (Table VIII) examined in 1937-38, Co 312 gave the highest yield but the lowest sucrose content, and had the highest ash content, hence a low saline coefficient.

In the following season, however, due to a higher sucrose and lower ash content the saline coefficient was the highest of all the varieties examined anywhere in the Punjab during the two seasons, although the figure was of the same order as that obtained at Meerut which provided the poorest quality canes in the United Provinces. The higher sucrose was due to the absence of *pyrilla*, but it is difficult to explain why a low ash content was obtained.

The average yield for the two seasons, however, was almost as good as that obtained at Karnal.

### *Rohat Harsana*

The soil of this village as shown below is a sandy loam and the lightest of all the soils examined in the Punjab during the survey :—

*Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available $P_2O_5$	Water-soluble salts	Clay	Silt	Sand
1st foot	0.0728	0.776	6.94	0.158	0.028	0.180	18.1	20.4	61.5
2nd foot	0.0560	0.484	6.28	0.182	0.032	0.220	23.1	19.5	57.6
3rd foot	0.0525	0.371	6.14	0.218	0.002	0.240	25.1	17.6	57.3

The soil is rich in total nitrogen, organic matter, exchangeable calcium and has an acid reaction.

Five varieties of cane were examined, viz. Co 213, Co 285, Co 301, Co 312 and Co 313 in the first season, and four in the second, viz. Co 244, Co 312, Co 313 and Co 331 (Table IX). In 1937-38 as a result of *pyrilla* none of the canes matured. Nevertheless, the outturn was very high, Co 312 with 815.5 maunds per acre, giving the best yield. The quality of the cane, however, was naturally very poor.

In 1938-39, all the four varieties examined matured early in December, the sucrose content of Co 331 (10.5 per cent) being the highest while that of Co 313 the lowest (9.7). The quality of cane resembled that grown at Karnal with a high ash content and a low saline coefficient. Yields were approximately 750 maunds per acre.

TABLE IX  
*Analytical data for cane varieties grown at Rohat Harsana*

Particulars	Percentage on cane						Glucose ratio	Purity coefficient	Saline coefficient	Yield per acre in maunds			Ripening period	
	Juice	Sucrose	Glucose	Total sugars	Total solids	Non-sugars				Ash	Cane	Sucrose		Total solids
Co 213 : 1937-38	71.9	7.7	0.62	8.3	9.9	1.6	0.646	8.1	79.3	11.9	570.0	40.5	50.4	11/2
Co 285 : 1937-38	68.3	7.1	0.50	7.6	9.4	1.8	0.707	7.0	78.9	10.0	700.0	60.8	73.5	11/2
Co 301 : 1937-38	73.5	8.5	0.77	9.3	10.8	1.5	0.680	9.1	72.0	12.5	775.0	65.8	83.7	14/2
Co 312 : 1937-38	72.8	8.8	0.56	9.4	10.8	1.4	0.465	6.4	81.5	18.4	815.5	71.7	88.1	2/2 to 24/2
1938-39	63.9	10.1	0.39	10.4	11.7	1.3	0.476	4.0	84.8	21.2	820.0	82.8	95.9	1/12 to 12/2
Co 313 : 1937-38	69.6	9.1	0.47	9.6	10.9	1.3	0.628	5.2	83.5	16.6	790.4	71.9	86.1	2/12 to 24/2
1938-39	60.1	9.7	0.30	10.0	11.6	1.6	0.457	3.1	84.0	20.7	760.0	73.7	88.2	1/2 to 12/2
Co 331 : 1938-39	63.1	10.5	0.26	10.8	12.2	1.4	0.415	2.5	85.8	23.1	690.0	72.4	84.2	1/12 to 12/2
Co 244 : 1938-39	62.6	10.4	0.279	10.7	12.1	1.4	0.470	2.4	85.9	22.1	780.0	81.1	94.4	1/12 to 12/2
Average : 1937-38	71.2	8.2	0.58	8.8	10.2	1.4	0.628	7.2	79.7	13.9	746.2	62.8	73.5	
1938-39	62.4	10.2	0.31	10.5	11.9	1.4	0.439	3.0	85.1	21.8	762.5	77.9	90.7	

TABLE X

*Analytical data for cane varieties grown at village Jaukhli*

Particulars	Percentage on cane						Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period	
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars				Ash	Cane	Sucro- se		Total solids
Co 244 : 1937-38	70.1	9.9	0.55	9.7	10.9	1.2	0.714	6.1	83.5	13.5	555.0	50.5	80.5	20/1 to 25/2
1938-39	66.1	10.3	0.21	10.5	12.0	1.5	0.610	2.3	86.1	21.4	600.0	61.8	72.0	7/12 to 19/2
Co 312 : 1937-38	73.3	8.7	0.65	9.4	10.4	1.0	0.432	7.5	82.5	20.4	628.3	54.7	66.0	17/2 to 25/2
1938-39	67.6	10.9	0.16	11.2	12.5	1.3	0.561	1.8	87.6	19.4	700.0	70.3	77.0	7/12 to 19/2
Co 313 : 1937-38	70.8	8.8	0.47	9.3	10.9	1.6	0.637	5.3	81.1	13.9	615.7	54.2	67.1	25/2
1938-39	64.5	10.0	0.20	10.2	11.7	1.5	0.648	1.9	85.0	17.3	650.0	65.0	76.0	7/12 to 19/2
Average : 1937-38	71.4	8.9	0.56	9.5	10.8	1.3	0.594	6.3	82.4	15.9	599.7	53.1	64.5	
1938-39	66.1	10.4	0.28	11.0	12.0	1.0	0.585	2.0	86.2	19.4	650.0	67.7	75.0	

*Jaukhli*

This is a typical village of the *khadar* tract, whose soil is a medium loam and characterized by a highly alkaline reaction, low exchangeable calcium and high water-soluble contents. The average composition is shown below :—

*Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available $P_2O_5$	Water-soluble salts	Clay	Silt	Sand
1st foot	0·0728	0·986	7·97	0·072	0·037	0·220	16·3	31·6	52·1
2nd foot	0·0588	0·807	8·11	0·074	0·014	0·220	20·1	37·2	42·8
3rd foot	0·0476	0·524	7·91	0·060	0·019	0·240	22·6	42·7	34·7

This soil had the lowest exchangeable calcium content of any of the soils examined in the Punjab—a fact which appears to be reflected in the ash content of the cane juice particularly in the case of Co 244 which was 0·71 per cent at Jaukhli but only 0·37 per cent at Tharu. Compared with other localities in the Punjab even in the abnormal season of 1937-38, Jaukhli produced only medium quality canes (Table X). In the following season all the three varieties matured early and had a moderately good sucrose content, a high ash content and a low saline coefficient, thus resembling the other canes in this area.

*Karnal*

Canes examined at Karnal were taken from the Government Agricultural Farm, which, as will be seen from the data below, has a clay loam soil rich in organic matter, total nitrogen and exchangeable calcium. The concentration of water-soluble salts, however, is rather high and the soil reaction acidic.

*Percentage on air-dried soil*

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available $P_2O_5$	Water-soluble salts	Clay	Silt	Sand
1st foot	0·0756	1·000	6·50	0·190	0·014	0·160	23·0	31·5	45·5
2nd foot	0·0483	0·936	6·42	0·220	0·037	0·110	29·1	29·2	41·8
3rd foot	0·0462	0·855	6·22	0·228	0·019	0·130	31·0	28·5	40·5

Of the four varieties examined in 1937-38, viz. Co 285, Co 312, Co 313 and Co 331 (Table XI), Co 312 and Co 331 were badly attacked by pyrrilla, yielding only 5 per cent and 4·5 per cent sucrose on cane, respectively. Co 312 gave the highest yield. All the four varieties had low purity coefficients and high glucose ratios, both the sucrose content and saline coefficients being low. The crop showed a tendency to lodge. In the following season all the four varieties examined, viz. Co 213, Co 312, Co 313 and Co 331 matured in the middle of December. The ash content of the juice was very high, but as regard composition, the cane was in no way inferior, to that on the Government Farm at Meerut, where improved methods of cultivation were followed. The sucrose content and ash were equal at both these localities, but the weight of cane produced per acre was higher at Karnal by 100 maunds than at Meerut.

#### GENERAL OBSERVATIONS

##### *Ripening of sugarcane*

It will be seen from Tables II-XI that the yield of stripped cane in the Punjab was about 40-50 per cent higher than in the United Provinces, which shows that the Punjab canes attained a more vigorous and greater vegetative growth than those in the neighbouring province. Hence, they must remain in the field for a longer period before they are fully mature, since all factors which increase vegetative growth cause delay in ripening. Again we see that the Punjab soils are richer in organic matter, total nitrogen and water-soluble salts and are much more heavily manured than the United Provinces soils. The number of irrigations given is also greater, but, taking into consideration the higher rainfall in the United Provinces, the total amount of water received by the sugarcane crop, both as irrigation and rain water, is usually the same. It is thus clear that, while most of the canes in the United Provinces will be fit for crushing early in the season, the same varieties will be unripe in the Punjab. If, therefore, the same varieties of cane are crushed in the two tracts at the same time, the United Provinces canes being fully ripe will naturally give a greater recovery.

##### *Quality and yield of sugarcane*

The data for the season 1937-38, relating to the quality and quantity of cane varieties which are common to the two tracts, are given in Table XII.

It will be seen from these figures, that apart from Co 331 which gave less total solids and sucrose per acre in the Punjab than in the United Provinces, all the varieties examined gave a greater outturn of stripped cane, sucrose and total solids in the Punjab. The quality of cane, however, as judged from the amount of sucrose and ash expressed as percentage on cane, purity and saline coefficient, was much poorer in the Punjab.

Similar data for the following season 1938-39 are given in Table XIII. It will be observed that this was a better season for cane both in the United Provinces and the Punjab, specially in the latter where the cane crop benefited from better climatic condition and was also free from pyrrilla, with the result that the sucrose percentage on cane was almost as good as in the United Provinces. Similarly the 'cane ratio' and net rendiment showed marked improvement. The yield of stripped cane per acre increased by over 130

TABLE XI  
*Analytical data for cane varieties grown at Karnal*

Particulars	Percentage on cane						Glu- cose ratio	Purity Saline coeffi- cient	Yield per acre in maunds			Ripening period		
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars			Ash	Cane	Sucro- se		Total solids	
Co 285 : 1937-38	75.5	7.7	1.44	9.1	10.1	1.0	0.505	18.7	76.9	15.5	613.3	47.2	61.9	27/12
Co 213 : 1938-39	65.0	10.0	0.36	10.4	12.0	1.6	0.579	3.8	83.1	17.3	650.0	65.0	78.0	9/12 to 23/2
Co 312 : 1937-38	68.5	5.0	1.50	6.5	7.7	1.2	0.358	30.0	65.8	13.8	757.3	37.8	58.3	28/2
1938-39	69.2	10.4	0.29	10.7	12.1	1.4	0.398	3.8	85.5	26.1	1265.0	131.6	155.1	9/12 to 23/2
Co 313 : 1937-38	67.2	7.9	0.76	8.7	9.7	1.0	0.420	9.7	81.9	18.8	612.1	48.3	59.3	28/2
1938-39	67.2	10.9	0.29	11.2	12.9	1.7	0.495	2.9	84.0	22.0	820.0	89.4	105.8	19/12 to 23/2
Co 331 : 1937-38	72.3	4.5	2.18	6.7	6.9	1.2	0.509	48.4	65.3	8.4	551.3	24.8	38.0	28/2
1938-39	66.6	10.0	0.48	10.5	11.9	1.4	0.462	3.5	83.5	21.8	1000.0	100.0	119.0	9/12 to 23/2
Average : 1937-38	70.9	6.3	1.47	7.8	8.6	1.1	0.448	26.7	72.5	14.1	633.5	39.5	54.4	
1938-39	66.9	10.3	0.34	10.6	12.2	1.6	0.481	3.5	84.1	21.8	934.0	96.5	114.0	

TABLE XII  
Average composition of sugarcane in the Punjab and the United Provinces and other relative data, 1937-38

Province	Percentage on sugarcane						Maunds per acre				
	Sucrose	Glucose	Total sugars	Total solids	Non-sugars	Ash	Purity coefficient	Saline coefficient	Stripped cane	Sucrose	Total solids
United Provinces	11.0	0.53	11.5	12.6	1.1	0.42	Co 213	87.0	303.0	33.0	37.6
Punjab	7.7	0.49	8.2	9.7	1.5	0.65		79.3	570.0	43.9	50.4
United Provinces	10.7	0.57	11.3	12.2	0.9	0.33	Co 244	87.4	437.1	46.1	53.0
Punjab	9.7	0.55	10.3	11.5	1.2	0.47		84.6	652.5	63.3	75.2
United Provinces	9.7	0.75	10.5	11.5	1.0	0.26	Co 312	84.1	559.2	53.0	60.1
Punjab	8.0	1.0	9.0	10.3	1.3	0.52		75.5	762.7	54.0	71.0
United Provinces	10.9	0.34	11.2	12.3	1.1	0.33	Co 313	83.5	474.6	67.9	57.9
Punjab	8.5	0.58	9.1	10.3	1.2	0.52		82.6	692.2	83.5	75.8
United Provinces	9.4	0.65	10.1	11.2	1.1	0.33	Co 331	83.6	533.0	49.4	56.7
Punjab	4.5	2.18	6.7	8.0	1.3	0.54		65.3	551.3	24.8	38.0

*Average relative data*

Particulars	United Provinces	Punjab
Sucrose per cent on cane . . . . .	10.3	7.7*
Ash per cent on cane . . . . .	0.342	0.539
Purity coefficient . . . . .	86.1	77.5
Saline coefficient . . . . .	30.1	14.3
Cane ratio . . . . .	11.7	19.2
Net rendiment or available sugar . . . . .	8.55	5.2
	Maunds	Maunds
Stripped cane per acre . . . . .	461.3	643.9
Sucrose per acre . . . . .	49.9	53.9
Total solids per acre . . . . .	53.4	63.3

\* A low figure due to pyrilla

maunds in the Punjab and by 150 maunds in the United Provinces, this latter increase being due to the fact that the figures for Bilari and Shahjahanpur, where large quantity of manure are generally applied, were included in the average for this season.

In considering the relative features which have been described in regard to the growth of cane obtained in the two provinces, both from the quantitative and qualitative aspects, the most important point perhaps to bear in mind is that the ash content and 'solids non-sugars' in the juice from the Punjab canes are considerably higher than in those from the United Provinces. This is, no doubt, due to the higher concentration of water-soluble salts in the Punjab soils, whereas in the United Provinces the soils are lighter and thus more permeable. The cane ratio or the number of tons of cane required to produce a ton of sugar is higher in the Punjab than in the United Provinces. This ratio is a function not only of the concentration of sucrose in the juice but also of its purity, the latter being the ratio of sucrose to total solids in the juice. The greater the proportion of mineral matter, the more difficult it is to recover sugar in the process of manufacture. Nitrogenous manuring tends to increase the percentage of impurities in the juice and consequently the cane ratio to a greater degree than might be expected solely from a consideration of differences in sucrose content. Consequently a relatively large percentage of sugar remains unrecovered in the factory when cane is grown under heavy manuring. However, some of these factors counterbalanced each other when comparing the sugar recovery from the canes of the western United Provinces and the south-eastern Punjab, and it was found on balance that the difference was not greater than about one per cent.

In order to gain more accurate data as to how far different nitrogenous manures affect the composition of cane juice, Co 312 was manured at the Shahjahanpur Sugarcane Research Station with 100 lb. and 200 lb. of nitrogen per acre in the form of castor-cake, ammonium sulphate and farmyard manure under two, four and six irrigations. The data from these investigations are given in Tables XIV-XVI, from which it will be seen that the sucrose content

TABLE XIII

*Average composition of sugarcane in the Punjab and United Provinces and other relative data, 1938-39*

Province	Percentage on sugarcane					Purity coefficient	Saline coefficient	Maunds, per acre			
	Sucrose	Glucose	Total sugars	Total solids	Non-sugars			Ash	Stripped cane	Sucrose	Total solids
United Provinces .	10.2	0.55	10.8	12.1	Co 213 1.3		0.332	32.2	397.0	40.2	48.5
Punjab . . .	10.0	0.37	10.4	12.0	1.6		0.567	17.6	610.0	61.0	78.0
United Provinces .	10.9	0.22	11.1	12.3	Co 214 1.2		0.286	38.1	400.0	43.6	49.2
Punjab . . .	10.7	0.23	10.6	12.1	1.5		0.479	21.7	723.0	75.2	87.5
United Provinces .	10.6	0.50	11.1	12.2	Co 312 1.1		0.267	39.7	722.0	76.3	87.8
Punjab . . .	10.5	0.29	10.8	12.1	1.3		0.488	21.5	909.0	95.4	110.0
United Provinces .	11.3	0.40	11.7	13.0	Co 313 1.2		0.331	34.1	578.0	65.3	75.1
Punjab . . .	10.3	0.27	10.6	12.1	1.6		0.511	21.1	757.0	78.0	91.6
United Provinces .	10.4	0.44	10.8	12.0	Co 331 1.1		0.290	35.9	764.0	79.4	91.7
Punjab . . .	10.3	0.30	10.6	12.0	1.4		0.475	21.7	885.0	91.1	106.2

*Average relative data*

Particulars	United Provinces	Punjab
Sucrose per cent on cane . . . . .	10·7	10·4
Ash per cent on cane . . . . .	0·314	0·504
Purity coefficient . . . . .	87·0	85·9
Saline coefficient . . . . .	34·1	20·6
Cane ratio . . . . .	11·4	12·0
Net rendiment or available sugar . . . . .	9·18	8·35
	Maunds	Maunds
Stripped cane per acre . . . . .	611·1*	776·2
Sucrose per acre . . . . .	63·9	78·8
Total solids per acre . . . . .	73·6	90·3

\* The average outturn was higher this season as Bilari and Shahjahanpur were included where large quantities of manure are applied

was depressed and ripening delayed as the quantities of nitrogen applied increased. No particular increase in the ash content of cane manured with ammonium sulphate was found, but the increase was significant when farmyard manure was employed. Generally speaking it may be stated with reasonable conviction that the amount of solids non-sugars in cane may be expected to increase in proportion to the amount of manure employed.

#### GENERAL CONCLUSIONS AS TO THE CAUSES RESPONSIBLE FOR THE ALLEGED INFERIOR QUALITY OF PUNJAB CANES COMPARED WITH UNITED PROVINCES CANES FOR SUGAR PRODUCTION

The general results of the survey indicate that the poor quality of the Punjab canes is mainly due to the composition of the soil of the province—a conclusion which has stimulated investigators to see to what extent the ash content of the juice can be lowered by altering the composition of the soil by the application of appropriate chemicals such as gypsum. There is tentative evidence already that gypsum may be efficacious for this purpose in heavy soils.

In regard to operations in sugar factories it will be seen from the appendix that the mineral content of the clarified juice is lowered to a greater extent if the carbonation process rather than the sulphitation process is employed, and as the Punjab has plenty of lime available this industry should flourish in certain localities provided judicious agricultural operations are followed, due notice taken of the likelihood of frost in the localities selected and the carbonation process followed in factories.

TABLE XIV  
*Sugarcane analysis*

(Sugarcane Experiment Station, Shahjahanpur, 1939)

Date of analysis	Variety of cane	Description of sample	Percentage on juice					Purity coeff. client	Saline coeff. client	Percentage on cane			Remarks	
			Glucose		Total solids	Non-sugars	Mineral matter			Glucose ratio	Juice	Sucrose		Total solids
			Sucrose	Glucose										
22/1	Co 312	Control N <sub>1</sub>	13.8	1.04	16.7	1.9	0.412	7.3	82.9	33.4	66.7	9.2	11.1	Six irrigations
"	"	Castor-cake N <sub>1</sub>	13.3	1.17	16.4	1.9	0.345	8.8	81.6	40.4	66.7	8.9	10.9	100 lb. nitrogen
"	"	" N <sub>2</sub>	12.6	1.65	16.1	1.9	0.345	12.9	78.0	38.6	67.7	8.5	10.9	200 lb. nitrogen
"	"	Control N <sub>1</sub>	14.8	0.64	17.3	1.9	0.430	3.9	86.1	38.5	67.7	9.9	11.5	
"	"	Ammonium sul- phate N <sub>2</sub>	13.5	1.59	16.5	1.6	0.300	10.2	80.9	55.1	68.6	9.3	11.5	100 lb. nitrogen
"	"	"	12.2	1.75	15.3	1.3	0.214	14.3	79.8	56.2	68.1	8.9	11.3	200 lb. nitrogen
"	"	Control	14.3	0.77	16.9	1.8	0.397	5.6	84.5	46.2	68.8	9.8	11.6	
"	"	F. Y. M. N <sub>1</sub>	13.9	0.90	16.6	1.8	0.360	6.2	83.8	43.1	66.6	9.3	11.1	100 lb. nitrogen
"	"	F. Y. M. N <sub>2</sub>	14.0	0.98	16.9	1.9	0.490	6.8	82.3	39.4	66.6	9.3	11.3	200 lb. nitrogen

TABLE XV  
*Sugarcane analysis*

(Sugarcane Experiment Station, Shahjahanpur, 1939)

Date of analysis	Variety of cane	Description of sample	Percentage of juice					Glucose ratio	Purity coeff. cent	Saline coeff. cent	Percentage of cane			Remarks
			Percentage of juice				Juice				Sucrose	Total solids		
			Sucrose	Glucose	Total solids	Non-sugars							Mineral matter	
23/1	Co 312	Control N <sub>1</sub>	14.7	1.03	18.1	2.4	0.325	7.0	81.4	45.5	65.0	9.6	11.8	Four irrigations
"	"	Castor-cake N <sub>2</sub>	12.7	1.63	16.0	1.7	0.443	12.9	79.0	28.5	65.6	8.3	10.5	100 lb. nitrogen
"	"	" N <sub>2</sub>	13.5	1.20	16.8	2.1	0.389	8.9	80.5	34.8	67.3	9.1	11.3	200 lb. nitrogen
"	"	Control N <sub>1</sub>	14.2	0.80	17.4	2.4	0.429	5.7	81.4	33.0	65.0	9.2	11.3	
"	"	Ammonium sulphate N <sub>2</sub>	12.7	1.58	16.1	1.8	0.399	12.4	79.4	35.6	66.6	8.5	10.7	100 lb. nitrogen
"	"	Ammonium sulphate N <sub>2</sub>	13.0	1.35	16.4	2.0	0.292	10.2	78.8	46.3	68.8	8.9	11.3	200 lb. nitrogen
"	"	Control N <sub>1</sub>	14.3	0.63	16.9	2.0	0.456	4.3	85.1	31.4	67.7	9.7	11.4	
"	"	F. Y. M. N <sub>2</sub>	13.6	1.0	17.1	2.5	0.451	7.2	79.1	30.0	64.3	8.7	11.0	100 lb. nitrogen
"	"	F. Y. M. N <sub>2</sub>	13.8	0.66	17.1	2.6	0.501	5.0	80.8	27.6	70.0	9.7	12.0	200 lb. nitrogen

TABLE XVI  
*Sugarcane analysis*

(Sugarcane Experiment Station, Shahjahanpur, 1939)

Date of analysis	Variety of cane	Description of sample	Percentage of juice					Percentage of cane			Remarks
			Sucrose	Glucose	Total solids	Non-sugars	Mineral matter	Glucose ratio	Purity coeff. cent	Saline coeff. cent	
24/1	Co 312	Control N <sub>1</sub>	14.7	0.76	17.5	2.0	0.430	5.2	84.4	34.3	Two irrigations
"	"	Castor-cake N <sub>1</sub>	13.8	1.38	17.2	2.0	0.236	10.0	79.8	58.4	100 lb. nitrogen
"	"	" N <sub>1</sub>	13.0	1.29	15.8	1.5	0.301	9.9	82.2	43.4	200 lb. nitrogen
"	"	Control N <sub>1</sub>	15.4	0.88	17.9	1.6	0.271	5.7	86.6	56.9	
"	"	Ammonium sulphate N <sub>1</sub>	12.0	1.31	15.2	1.9	0.292	8.9	79.4	41.1	100 lb. nitrogen
"	"	Ammonium sulphate N <sub>1</sub>	14.0	1.02	17.0	2.0	0.280	7.3	82.2	49.7	200 lb. nitrogen
"	"	Control N <sub>1</sub>	13.0	0.96	15.7	1.7	0.369	7.4	83.5	35.4	
"	"	F. Y. M. N <sub>1</sub>	16.0	0.69	18.2	1.5	0.433	4.5	85.1	35.1	100 lb. nitrogen
"	"	F. Y. M. N <sub>1</sub>	14.7	0.63	17.5	2.1	0.438	4.0	83.8	33.6	200 lb. nitrogen

Another point of considerable importance brought out during this survey is the fact that, although manuring results in an increased yield of cane, it tends to lower the quality of the juice, and it appears, therefore, that measures designed to increase the yield of cane should be carefully controlled in conjunction with the quality of cane obtained so as to ensure that the latter is not adversely affected.

The quality and yield of the cane crop depend mainly on : (i) climate, (ii) nature of soil and (iii) agricultural operations. It is a well-known fact that in order to produce one pound of dry matter in an arid region more water will be required than to produce the same amount of crop in a humid climate. If we assume, therefore, the concentration of the soil solution (in more general terms the amount of water-soluble salts present in the soil) to be the same in both the cases, it is likely that the crop grown in a dry climate will take up and retain a greater amount of plant food material owing to increased transpiration.

Again, the concentration and nature of the soil solution will depend respectively upon the amount and nature of soluble salts present in any particular soil. Furthermore, the application of manures as followed in the Punjab without a corresponding increase in the amount of water applied appears to be responsible for an increase in the amount of mineral matter in the cane crop. Considering all the factors, it appears that the greater amount of ash present in the juice from the Punjab canes is the main cause of their alleged inferiority. This is confirmed by the following figures (1937-38) relating to Sonapat in the Punjab where the cane crop was raised under ordinary *zenindari* conditions, and Bisokhar and Meerut in the United Provinces, where improved methods of cultivation depending upon liberal irrigation and application of sufficient manures were followed.

It will be seen from the figures of purity and saline coefficients for different varieties of cane grown at various stations in the two provinces (Tables XII and XIII) that the canes from the western United Provinces are generally superior to those obtained from the south-eastern Punjab. Nevertheless, as shown above, those from Bisokhar and Meerut were not as good as those from Sonapat, because the latter were grown under improved methods of cultivation.

It seems that attempts to increase the yield of cane beyond a certain limit are likely to result in deterioration of quality unless suitable varieties of cane can be evolved which will maintain their quality as quantitative production

*Percentage on cane*

Variety	Locality	Juice (per cent)	Sucrose	Glucose	Total solids	Ash	Glucose ratio	Purity	Saline coefficient
Co 313	Sonapat . . .	62.2	12.0	0.1	13.4	0.390	1.0	89.8	30.8
„	Bisokhar . . .	60.0	11.5	0.15	13.2	0.528	1.3	86.9	21.7
„	Meerut . . .	60.0	10.6	0.23	11.8	0.501	2.1	90.1	21.2
Co 331	Sonapat . . .	65.6	10.6	0.30	12.1	0.360	3.0	87.6	29.5
„	Bishokhar . . .	71.1	9.6	0.55	11.6	0.404	5.6	82.8	23.8
„	Meerut . . .	69.3	9.4	0.36	11.2	0.479	3.8	83.8	19.5

is increased. In this connection mention may be made of an interesting observation made at Amroha, which produced the best canes of any in the United Provinces or the Punjab. It was found that the amount of mineral matter which the cane crop is able to absorb during its growth reaches a maximum at maturity, and if the crop remains longer in the field the mineral matter either remains stationary or decreases. In other words, once the crop is mature, there is little likelihood of any further increase in the mineral content of the juice. Obviously, in this respect early-maturing varieties will have an advantage over those which mature late, although their yields must naturally be low. Attempts should be made to evolve varieties which will give improved yields and yet mature early. Further, such varieties should maintain their quality over the entire crushing period as long as they remain standing in the field.

### SUMMARY

It was found during the sugarcane seasons 1935-36 and 1936-37 that the recovery of white sugar at the Sonepat Sugar Factory in the south-eastern Punjab from sugarcane grown in the surrounding areas was only 7.7 per cent, whereas cane imported from the neighbouring tracts across the river Jumna in the United Provinces gave a recovery of 10.18 per cent. The present investigation was conducted with a view to ascertain the causes responsible for this wide difference.

A number of localities were selected in each of the two tracts and the same varieties in each were analysed during the sugarcane seasons 1937-38 and 1938-39. The soil from the fields growing these canes was sampled at a number of places to a depth of 3 ft. and analysed for different constituents.

It was found that the composition of the soil in the two tracts differs widely in the matter of organic matter, nitrogen and water-soluble salts content, the Punjab soils being richer in these constituents. The United Provinces soils are inclined to be slightly more acidic and lighter than the Punjab soils.

The analysis of different varieties of cane show that canes grown in the Punjab have a slightly lower sucrose and glucose content but contain more mineral matter in the juice than corresponding canes in the United Provinces. These differences are reflected in the higher purity coefficient and significantly superior saline coefficient of the cane from the United Provinces.

It has been found that the higher mineral matter in the juice of the Punjab canes is the main reason for the low recovery of white sugar in the Punjab.

It has been shown that the mineral matter in the juice of sugarcane does not increase after the canes have reached maturity. On the other hand in certain cases the mineral content has been found to decrease after maturity. This indicates that early ripening varieties are better suited for the soil and climatic conditions of the Punjab. Efforts should, therefore, be directed to evolve such varieties and at the same time aim for higher yields.

The carbonation process is better suited than the sulphitation process for the manufacture of white sugar in the Punjab as the former reduces the mineral content of the mixed juice during clarification to an extent which is almost four times as great as that obtained with the other,

## ACKNOWLEDGEMENTS

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## REFERENCE

Lander, P. E. and Ramji Narain. (1936). Mineral matter in the juice of sugarcane and its effect on the recovery of white sugar, I. *Indian J. agric. Sci.* **6**, 1218

## APPENDIX A

In the manufacture of white sugar two of the most important methods employed for the clarification of sugarcane juice in the factory are : —(i) the sulphitation process, (ii) the carbonation process. In the first process lime and sulphur are used as clarifying agents and in the second carbon dioxide and lime. The latter process is more expensive than the former on account of the greater quantity of lime used but the increase in cost is compensated for by a higher recovery of sugar of better quality. Since the juice of sugarcane in the Punjab is characterized by the presence of mineral matter in relatively high amounts, the process of clarification which will yield clarified juice with a lower mineral content will naturally be better suited to conditions in the Punjab. Data were collected during the survey to ascertain which of the two processes is more suited for the Punjab canes. Two factories working within a few furlongs of each other were selected for acquiring these data and the figures obtained are given below :—

*Sulphitation process*

Particulars	Brix	Pol	Purity	
Mixed juice . . . .	15.5	11.7	75.7	} Average of the 1st fortnight
Clarified juice . . . .	17.1	13.2	77.0	
Mixed juice . . . .	16.0	12.2	76.5	} Average of the 2nd fortnight
Clarified juice . . . .	18.2	14.2	78.1	
Mixed juice . . . .	16.3	12.6	77.3	} Average of the 3rd fortnight
Clarified juice . . . .	18.8	14.8	78.6	
Mixed juice . . . .	16.6	13.1	78.9	} Average of the 4th fortnight
Clarified juice . . . .	18.1	14.4	79.7	

Average increase of purity from mixed juice to clarified juice is equal to 1.25

*Carbonation process*

Particulars	Brix	Pol	Purity	
Mixed juice . . . .	15.0	11.3	75.2	} Average of 1st fortnight
Clarified juice . . . .	13.4	10.5	78.0	
Mixed juice . . . .	15.9	12.1	76.3	} Average of 2nd fortnight
Clarified juice . . . .	14.3	11.3	78.2	
Mixed juice . . . .	16.4	12.6	77.1	} Average of 3rd fortnight
Clarified juice . . . .	15.3	12.2	79.3	
Mixed juice . . . .	16.5	12.9	78.3	} Average of 4th fortnight
Clarified juice . . . .	15.5	12.6	80.9	

Average increase of purity 2.4

These figures indicate that there is a greater elimination of non-saccharine solids from the juice by the carbonation process than with the other. The following figures further show that the elimination of ash is also much greater by the former process, being almost four times as great as in the latter.

Particulars	Ash per cent juice
<i>Sulphitation process</i>	
Mixed juice . . . . .	0.530
Clarified juice . . . . .	0.474
Mixed juice . . . . .	0.565
Clarified juice . . . . .	0.487
<i>Carbonation process</i>	
Mixed juice . . . . .	0.540
Clarified juice . . . . .	0.317
Mixed juice . . . . .	0.548
Clarified juice . . . . .	0.275

These data show that the carbonation process is better suited for Punjab canes than the sulphitation process

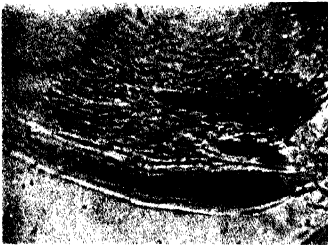




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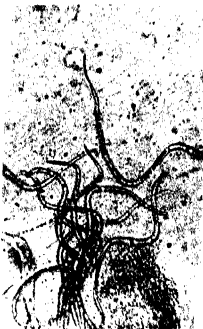
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For explanation see page 742

# NOTES ON SOME FUNGI ISOLATED FROM 'BLACK POINT' AFFECTED WHEAT KERNELS IN THE CENTRAL PROVINCES

BY

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(With Plate XXIX and eight text-figures)

**I**N a previous publication [Dastur, 1933] it was mentioned that from 'black point' infected wheat kernels, though they all look alike, more than one kind of fungi have been isolated when incubated under aseptic conditions. This paper deals with a study of some of those fungi which have not been as yet recorded on wheat kernels.

The 'black point' infected seed was surface-sterilized by a brief soak either in a 0.1 per cent solution of corrosive sublimate or in rectified spirit before it was planted on moist sterilized filter paper in a sterilized petri dish.

In a majority of cases not more than one fungus at a time was found associated with the diseased seed. In those few cases which gave a mixed fungus flora the mixture included one or more of the following :—*Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Fusarium* sp., *Chaetomium* sp., *Alternaria* sp., *Cladosporium* sp.

*Cochliobolus tritici* n. sp. Dast.

In some rare cases an ascigerous fungus was isolated from 'black point' affected kernels ; 'black point' affected seeds are usually viable ; but those in which the blackening of the embryo end was caused by this ascigerous fungus failed to germinate ; as the number of isolations of this fungus has been very small it is not possible to conclude that the kernel diseased by this fungus is always not viable. A few days after the surface-sterilized seed was planted on filter paper in a petri dish under sterile conditions minute wartlike protuberances appeared on the seed. Later the pericarp ruptured and globose or sub-globose black bodies, the perithecia, became distinctly visible ; similar bodies were also seen scattered amongst the hyphae which had spread from the diseased seed to the filter paper. No other organism was found associated with this fungus. On the filter paper the perithecia are scattered ; on the seed they are at first scattered but later they become crowded together forming a carbonaceous, brittle, crust-like mass. The perithecia on the kernels are not embedded in its tissues but are formed on the outside of the pericarp partially surrounded by a cob-web of dark coloured hyphae. There is no development of a stroma (Plate XXIX, fig. 1). On agar media and sterilized wheat stalks, seed or bran the perithecia are similarly developed ; they are erumpent, and scattered or gregarious. They have generally a prominent neck especially those formed on the 'black point' affected kernel (Plate XXIX, fig. 2), but at times the neck is absent. This is particularly so in the case of perithecia

developed on culture media (Plate XXIX, fig. 3). They are globose, sub-globose or flask shaped, usually glabrous; but at times there is a development of undifferentiated hyphae; the perithecia are black in colour; the neck when present is cylindrical and has a fringe of hyaline cells at the tip. The walls of the perithecia are thick but fragile; they readily break even under slight pressure. The perithecia with the beak measure  $219.8-596.6 \times 172.1-406.1\mu$ ; the body measures  $204-550\mu$ ; the neck measures  $53.1-235.9 \times 53.1-123.9\mu$ .

The asci are numerous and embedded in filiform paraphyses (Plate XXIX, fig. 4). In a mass, asci and paraphyses have a greenish tinge but individually they are hyaline. The asci are usually long and narrow (Plate XXIX, fig. 5) but at times they are short and broad; they are straight or slightly curved; they are more or less clavate in shape; the long asci are usually pedicellate (Plate XXIX, fig. 6); whereas the short ones are sessile with a rounded base (Plate XXIX, fig. 7); they measure  $79.8-228 \times 13.3-34.2\mu$ ; usually they measure  $121.6-203.2 \times 15.2-22.8\mu$ . They contain eight ascospores.

Paraphyses are generally inconspicuous, though numerous, and surround the asci; they are very slender, upto  $1.7\mu$ , in width; they are frequently branched, occasionally dichotomously; they are multiseptate; not constricted at the septum; the apex is rounded and slightly broader than the rest of the body of the paraphysis.

The ascospores are coiled in a close helix (Plate XXIX, fig. 8); they usually escape from the asci through an opening formed at the apex by the dissolution of the apical part (Plate XXIX, fig. 5). At times they escape from the basal end of the ascus, and occasionally from both the ends simultaneously. As the helix of the ascospores emerges through the opening it gets uncoiled; when the whole mass of coiled ascospores is out of the ascus they are usually completely free from each other and are scattered some distance away from the ascus. At times only a few ascospores escape from the helix and the remaining ascospores are still confined in a loose helix. The ascospore is thin, long and helioid or horse-shoe or wavy or contorted in shape (Plate XXIX, fig. 9); rarely straight; and, therefore, its length cannot very accurately be measured; the apex is slightly rounded; the basal part tapers gradually and the end is pointed; it is hyaline in colour, but rarely has an olivaceous tinge; it is many septate,  $4-12$ ; at times there is a constriction at the septum; the ascospores measure  $125.4-301.6 \times 3.8-7.6\mu$  they germinate readily; germ-tubes are developed from any or all segments.

In one of the progenies of a culture started from a single ascus, conidia resembling those of *Helminthosporium* were developed. These conidia are light brown or honey coloured, and 5 to 9 septate. In shape they are generally elliptical and straight or slightly curved; they are not variable in shape. They are rounded at both ends, have a basal scar and are never forked. They measure  $45.6-83.6 \times 11.4-15.2\mu$ . The germination is bi-polar; the wall is firm.

Single spore cultures of this *Helminthosporium* gave only the conidial stage on Glucose agar and Rice meal agar; on two per cent plain agar slants conidia were not developed; but only empty globular or flask shaped bodies black or brown in colour, resembling the perithecia described above, were

formed ; on sterilized wheat grains and wheat bran perithecia and conidia were developed.

Conidia from a single spore culture of this *Helminthosporium* were used for inoculating ears of wheat plants grown in pots. The inoculum was placed on glumes after the flowers had set. The glumes of the grains of the inoculated spikelets showed typical symptoms of infection. The glumes had the characteristic tobacco coloured or blackish brown coloured lesions and the kernels showed the typical 'black point'. When these glumes and kernels were planted under aseptic conditions on culture media the ascigerous fungus was isolated. The asci measured  $126.0-231.0 \times 15.75-21.0 \mu$  ; the ascospores measured  $157.5-345.4 \mu$ .

Surface-sterilized wheat and rice grains were inoculated with this single-spore culture of *Helminthosporium*, and incubated in sterilized moist chambers. The perithecial stage was not developed but the hyphae produced *Helminthosporium* conidia in large numbers. The conidia on these wheat grains measured  $38.0-83.6 \times 11.4-15.2 \mu$  ; the number of septa varied from five to eleven. The conidia on rice grains were similar to those found on cultures of the ascigerous fungus and measured  $38.0-58.8 \times 7.6-15.2 \mu$ . The septa varied from five to nine.

#### TAXONOMY

The genus *Ophiobolus* Riess, in the broad Saccardian sense, can be readily divided into two distinct series, the helicoid and the non-helicoid ascigerous series according to the arrangement of the ascospores in the asci. The known perfect stages of the graminicolous *Helminthosporia* belong to the helicoid series. Drechsler [1934] has shown that these graminicolous *Helminthosporia* have characteristics which are sufficiently distinctive and constant to be grouped together for purposes of classification. He, therefore, has removed this helicoid series from the original genus *Ophiobolus* and has placed it in a new genus, *Cochliobolus*, a name which indicates the helicoid arrangement of the ascospores. The type species is *C. heterostrophus* (Syn. *Ophiobolus heterostrophus* Drechs.), the ascigerous stage of *Helminthosporium maydis* Nishikado et Miyake on *Zea mays*. Therefore, according to Drechsler, the following members of the helicoid ascigerous series, *Ophiobolus miyabeanus* Ito et Kuribayashi (Syn. *H. Oryzae*. Breda de Hann) on *Oryza sativa* ; *O. sativus* (P. K. et B.) Ito et Kuribayashi (Syn. *H. sativum* Pammel, *H. acrothecioides* Lindfors) on *Hordeum sativum* Jess. and *Triticum vulgare* ; *O. setariae* Sawada Ito et Kuribayashi (Syn. *H. setariae* Sawada) on *Setaria italica*, *S. glauca* and *S. viridis* ; *O. kusanoi* Nishikado (Syn. *H. kusanoi* Nishikado) on *Eragrostis major* would now be renamed *Cochliobolus miyabeanus* (Ito et Kuribayashi) Drechs., *C. sativus* (Ito et Kuribayashi) Drechs., *C. setariae* (Ito et Kuribayashi) Drechs. and *C. kusanoi* (Nishikado) Drechs. respectively. The ascigerous stage of *Helminthosporium stenospilum* Drechs. on *Saccharum officinalis* belongs to the helicoid series and has been named *Cochliobolus stenospilus* by Matsumoto and Yamamoto [1936]. Our fungus, both in its conidial and perfect stages, provides a close parallelism with these six species. There is a marked similarity between the perithecia of these species and our fungus. They are globose or flasked shaped, black or blackish brown in colour and have a thick pseudo-parenchymatous fragile wall ; the beak of the

perithecium is without setae as in *C. miyabeanus* and *C. heterostrophus*; judging from the illustration given by Matsumoto and Yamamoto [1936] the beak of the perithecium of *C. stenospilus* also seems to be without setae, the body is glabrous or may occasionally bear sterile hyphae. The perithecium may be with or without a beak. There is a considerable difference in the size of the perithecia of these seven species (Table I).

TABLE I

*Size of perithecia and their ostiolar beaks*

	Perithecia	Ostiolar beak
<i>C. miyabeanus</i> . . .	370—760 × 370—780 $\mu$	95—200 × 55—110 $\mu$
<i>C. sativus</i> . . .	770—530 × 340—470 $\mu$	90—150 × 80—110 $\mu$
<i>C. setariae</i> . . .	240—500 × 220—315 $\mu$	60—125 × 50—110 $\mu$
<i>C. heterostrophus</i> . . .	400 × 400—600 $\mu$	150 × 150 $\mu$
<i>C. kusanoi</i> . . .	300—350 × 300—350 $\mu$	.. ..
<i>C. stenospilus</i> . . .	266—462 × 238—448 $\mu$	.. ..
<i>C. n. sp.</i> . . .	220—597 × 172—406 $\mu$	53—236 × 56—124 $\mu$

The asci have a general resemblance in size; the range of variation both in the length and breadth of the asci of our fungus is much greater than that of other species (Table II).

TABLE II

*Size of asci and ascospores*

	Asci		Ascospores		
	Length	Width	Length	Width	Septation
<i>C. miyabeanus</i> . .	142—235 $\mu$	21—36 $\mu$	235—468 $\mu$	6—9 $\mu$	6—16
<i>C. sativus</i> . . .	110—220 $\mu$	32—45 $\mu$	160—360 $\mu$	6—9 $\mu$	6—13
<i>C. setariae</i> . . .	130—150 $\mu$	22—32 $\mu$	200—315 $\mu$	6—7 $\mu$	5—9
<i>C. heterostrophus</i> . .	160—180 $\mu$	24—28 $\mu$	130—340 $\mu$	6—7 $\mu$	..
<i>C. kusanoi</i> . . .	130—170 $\mu$	14—18 $\mu$	140—170 $\mu$	5 $\mu$	6—8
<i>C. stenospilus</i> . .	127—195 $\mu$	20—33 $\mu$	130—300 $\mu$	6—8 $\mu$	4—12
<i>C. n. sp.</i> . . .	80—228 $\mu$	13—34 $\mu$	125—301 $\mu$	4—8 $\mu$	4—12

The smallest ascus of our fungus is much smaller than the smallest of the other species ; but the maximum measurement is very close to that of *C. miyabeanus* and *C. sativus*. In width the smallest measurement is practically the same as that of *C. kusanoi* and the maximum is very close to that of *C. miyabeanus*, *C. setariae* and *C. stenospilus*.

In the number of ascospores in an ascus our fungus resembles *C. kusanoi* ; both have invariably eight ascospores. In *C. heterosporus* the number varies from one to four (typically 4) and in the remaining four species it varies from one to eight.

The range of variation in the length of the ascospores of our fungus is practically the same as that of *C. stenospilus* ; the ascospores of the other species except those of *C. kusanoi* are longer than our fungus. There is no marked difference in the width of the ascospores of these species ; the number of septa of the ascospores of our fungus is the same as that of *C. stenospilus* and the ascospores of both are mostly flagelliform.

The conidia are brown or brownish in colour fusiform or long elliptical in shape, occasionally slightly curved, five to nine septate measuring  $45.6-83.6 \times 11.4-15.2 \mu$  ; they have never been observed to be forked ; their wall is firm ; hilum is present. The germination is bipolar, germ tubes have not been seen to arise from the intermediate cells.

Our fungus does not possess complete similarity with any one of the known species of the helicoid series of the ascigerous stage of the graminicolous Helminthosporia ; though in some individual characters it may resemble one or more of these known species. Our fungus is, therefore, considered to be a new species of the helicoid series. It is congeneric with *Cochliobolus* and the binomial *C. tritici* sp. n. is proposed.

*Cochliobolus tritici* sp. n. Dastur

Perithecia scattered or gregarious, black or brownish black, pseudoparenchymatous, fragile, flask shaped, with or without ostiolar beak ; bodies globose,  $220-597 \times 172-406 \mu$  ; usually glabrous, at times covered with vegetative hyphae ; beaks, when present, well developed, cylindrical,  $53-236 \times 53-124 \mu$  ; asci numerous cylindrical or clavate, straight or slightly curved widest below the middle, rounded at the apex ; shortly stipitate at the base or sessile hyaline and thin walled  $80-228 \times 13.0-34.0 \mu$ . Paraphyses numerous, hyaline, at times dichotomously branched, extremely fine, upto  $1.66 \mu$  wide, septate. Ascospores, eight in number, disposed in a strongly helicoid arrangement, flagelliform or filiform, obtusely pointed at the apex and sharply pointed at the base ; wider at the apical portion than the basal which is tapering ; four to twelve septate hyaline in colour  $125.4-301.6$  by  $3.8-7.6 \mu$ . Conidia straight or slightly curved, elliptical with broadly rounded ends, five to nine septate ; basal scar present, wall firm light brown to honey coloured,  $45.6$  to  $83.6$  by  $11.4$  to  $15.2 \mu$  ; germination bi-polar.

Hab. on kernel of *Triticum vulgare*.

*Cochliobolus tritici*, sp. nova

Perithecia dispersa vel aggregata, nigra vel brunneo-nigra, pseudoparenchymatica, fragilia, amphorae similia, ostiolari rostro praesente vel absente ; corpora globosa,  $220-597 \times 172-406 \mu$  ; generatim glabra, non raro tamen

operta hyphis vegetativis; rostra, si adsunt, bene evoluta, cylindrica,  $53-236 \times 53-124\mu$ ; asci plures cylindrici vel clavati, recti vel leviter curati, latiores sub medio, rotundi in apice; sessiles vel breviter stipitati in basi, hyalini, et tenuibus parietibus praediti, magnitudinis  $80-228 \times 13.0-34.0\mu$ . Paraphyses plures, hyalinae, non raro dichotome ramificatae, admodum graciles, latitudinis ad  $1.66\mu$ , septatae. Ascosporae numero 8, dispositione valde helicoidali ordinatae, flagelliformes vel filiformes, obtusae in apice, valde acutae in basi; latiores ad apicem quam ad tenuescentem basim;  $4-12$  septatae, colore hyalinae, magnitudinis  $125.4-301.6 \times 3.8-7.6\mu$ . Conidia recta vel leviter curvata, elliptica, extremitatibus late globata,  $5-9$  septata; cicatrix basalis adest; parietes firmi, colore ex tenuiter brunneo ad melleum praediti,  $45.6-83.6 \times 11.4-15.2\mu$ ; germinatio bipolaris.

Habitat in seminibus *Triticum vulgare*.

Type specimens are deposited in the herbaria of the Mycologist to the Government of the Central Provinces and Berar and of the Imperial Mycologist, New Delhi.

This fungus was isolated some years back from 'black point' affected wheat kernels. During the writer's absence on leave most of the cultures of fungi isolated from this source were lost as a result of a bad infection by mites. Since then innumerable 'black point' affected wheat kernels have been planted on agar media and moist filter papers but from none of these plantings this fungus has been obtained, though various other fungi previously secured have been re-isolated. Several methods for the surface sterilization were adopted including the use of chemicals such as silver nitrate which Davies [1935] has found to be less toxic than mercuric chloride.

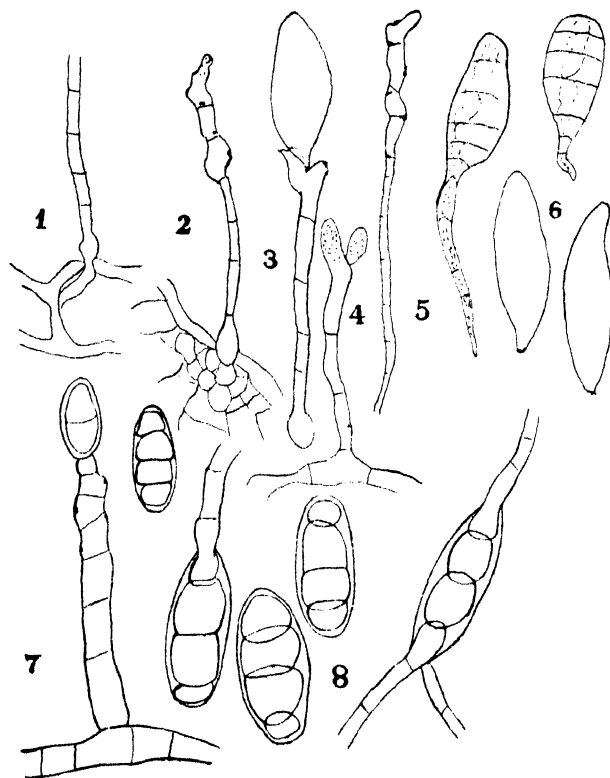
#### *Helminthosporium* sp. A

The growth of this fungus on the infected seed is characteristic; it can be readily differentiated from that of other fungi growing on 'black point' kernels when incubated under moist conditions. The aerial mycelium is scanty, both on the pericarp of the kernel and on the filter paper on which the kernel is planted. Both the pericarp and the filter paper are covered with a black or sooty powdery mass the conidia and conidiophores.

#### Conidiophores

Conidiophores on the kernel are developed in two ways; either directly from the mycelium in the cells of the host tissue or from the thin layer of aerial mycelium developed on the outside of the pericarp. The mycelium in the pericarp generally forms a stroma from which the conidiophore arises; but the conidiophore may also arise directly from a hypha in the sub-epidermal cells. When the conidiophores arise directly from the mycelium in the tissues of the kernel, the lower end of the conidiophore is swollen or bulbous (Figs. 1-3). But when the conidiophore is borne on the aerial mycelium it is a prolongation of a hyphal branch (Figs. 4 and 5); the beginning of the development of this conidiophore is marked by slight thickening, swelling and colouring of the terminal cell of this hypha. The conidium is unbranched; it usually arises from the substratum singly and is coloured light brown. The number of conidia borne on conidiophores arising from the pericarp of the kernel is small, one to seven, but of those borne on conidiophores in cultures is as much as 17 judging from the number of scars and geniculations present on

conidiophores. The development of conidia is typical of the genus *Helminthosporium*. The conidium is borne terminally; just below the point of its attachment the conidiophore grows onward forming a geniculation and bears another conidium. The process continues making the conidiophore geniculated; the number of bends correspond with the number of conidia developed.



*Helminthosporium* sp. A. ( $\times 270$ )

- FIG. 1. A conidiophore emerging through an epidermal cell  
 FIG. 2. A conidiophore developing from a stroma  
 FIG. 3. A conidiophore with a conidium  
 FIG. 4. A conidiophore arising laterally from a hypha  
 FIG. 5. A hypha developing into a conidiophore  
 FIG. 6. Conidia, two conidia are germinating

*Helminthosporium* sp. B. ( $\times 600$ )

- FIG. 7. A conidiophore developing laterally from a hypha  
 FIG. 8. Conidia, two conidia are germinating

The conidiophores borne on the pericarp measure  $41-243\mu$  up to the first scar or geniculation; their bulbous ends are  $5.5$  to  $11\mu$  in width. The number of septa from the bulbous end up to the first scar varies from three to six.

### *Conidia*

The conidia both on the wheat kernel and on culture media vary in shape ; they are obpyriform, obovate spear-head shaped or elongated elliptical ; the apex is either broadly rounded or pointed ; there are variations between these two extremes ; the conidia are stipitate and have a conspicuous hilum ; the stipe is about 3—9 $\mu$  long ; they are straight and regular but at times are crushed out of shape as they are crowded together ; the mature conidia are dark brown or honey coloured ; the septa are indistinct (Plate XXIX, fig. 13) ; they vary from three to seven ; at the basal end there is a distinct hyaline or lighter coloured area ; the wall is firm.

The germination is invariably from the basal end or the hilum (Fig. 6). The conidia measure 45.6—91.0  $\times$  18.7—30.0 $\mu$  generally 52.0—78.0  $\times$  18.7—30.4 $\mu$ .

In culture media the growth of this *Helminthosporium* is as characteristic as on the 'black-point' kernel. The aerial growth is limited ; the mycelium forms a thin felt-like growth ; when the conidia develop the colour becomes greenish black.

Inoculations of wheat seedlings failed to produce lesions on the leaves or stem ; but when immature ear heads were inoculated the glumes developed lesions and the kernels the typical smudge on the embryo end.

### *Helminthosporium* sp. B.

This *Helminthosporium* differs from the other *Helminthosporia* isolated from 'black point' affected wheat kernels in the conidiophores and conidia being very small, and in the conidia having a constant number of septa, namely three.

The conidiophores are very sparsely developed directly from the tissues of the kernel ; they emerge singly between the epidermal cells of the pericarp ; they are scattered ; their basal segment is not swollen. The incubated kernel on its outside is covered by a layer of brown mycelium from which conidiophores are developed in large numbers. They are generally formed laterally but in some cases they are borne terminally, the brown hypha bearing a conidium at its apex. The conidiophores that emerge from the host tissues or are borne laterally from the hyphae of the mycelial felt are short, unbranched, light to dark honey coloured ; the head bearing the conidia is very slightly broader than the rest of the conidiophore ; it is not strongly geniculated at the points of attachment of the conidia ; the scars marking these points are close to each other (Fig. 7) ; up to the first scar the conidiophores have three to five septa and measure 23.0—53.2  $\times$  3.5 $\mu$  ; the number of conidia borne is small, about two to six.

The conidia are elliptical in shape, both ends are similar and broadly rounded ; the basal end is distinguished by a not too prominent hilum ; the conidial wall is smooth, firm and thick, light to dark honey coloured ; the septa are three and clearly visible ; the germination is from each end, never from intermediate cells ; usually it is from the hilum end that the germ-tube first develops ; only one germ-tube is developed from each end ; but from the base of the lowermost cell a side branch is often developed (Fig. 8). The conidia measure 18.75—30.0  $\times$  7.5—11.25 $\mu$ .

This *Helminthosporium* is not the same as *H. triseptum* Drechs. isolated from velvet grass, *Notholcus lanatus* by Drechsler [1923]. *H. triseptum* has

bigger conidia,  $35-50 \times 15-21\mu$ ; they are dark olivaceous in colour and germinate only from the basal end.

The coleoptile and stems of seedlings inoculated with this fungus develop tobacco brown lesions; the roots turn brown. 'Black point' affected kernels were developed from inoculated flower heads.

*Pseudophoma* sp.

This disease is first noticed on wheat ears. The infected immature heads are lighter green in colour than the healthy heads; this difference in colour is noticeable only when the ear is green; when it matures and turns brown the colour of the infected ear is the same as that of the healthy ear. A few or all the spikelets in a ear may be affected. The infection is first seen on the outer palae; it commences as a minute pale brownish speck; it enlarges elliptically along the length of the palae forming a diffused lesion and ultimately may cover the greater part of the palae; the diseased area later turns tobacco brown; at a later stage the central part of the lesion turns lighter in colour, pale straw coloured or slivery grey coloured; the lesion thus has a distinct dark brown margin, the outline of which is not sharply defined. In the pale coloured centre black pycnidia are developed; they are not scattered but are arranged in rows between the vascular strands or veins. The infection may spread to the inner membranous palae; the lesion is diffused and tobacco brown in colour; pycnidia in linear rows are developed on the inner palae as well. The kernel may be well developed or shrivelled or aborted. The infected kernel does not necessarily have a black smudge at the embryo end; the lesion is usually a brown coloured line on the furrow, other parts of the pericarp may also be affected.

Transverse sections of a glume or a pericarp through a lesion with pycnidia show that they may originate in cells just below the epidermis, so that the mature pycnidium looks as if it had developed superficially, or they may develop in the inner tissues, in which case the mature pycnidium fills practically the whole thickness of the glume or the pericarp (Plate XXIX, figs. 10 and 11). In the host tissues the pycnidia do not seem to be embedded in a stroma (Plate XXIX, figs. 10 and 11). The pycnidia burst through the epidermis. When 'black point' affected kernels are planted on moist filter paper and incubated in moist chambers under aseptic conditions at room temperature the fungus does not develop a prominent growth of the aerial mycelium; it is scanty and spreads out fan-like on the filter paper; the colour is brownish or blackish brown. On the filter paper the pycnidia are formed singly, are scattered and superficial; there is no trace of the presence of a stroma. On the incubated kernel also the mycelial growth is very scanty and appressed to the pericarp. The pycnidia may be crowded together but there is no development of a stroma. On agar media also pycnidia are without a stroma. They are thick walled, coriaceous to carbonous, pear shaped or sub-globose, and bear a short but distinct beak (Plate XXIX, fig. 12). When seen from above under high magnification a distinct ostiole or opening is visible; the ostiole is not minute; basidia are absent or obsolete; the pycnidia measure  $38-53 \times 152-228\mu$ . The conidia escape from the ostiole in a long tendril or worm-like mass. They are hyaline elliptical or ovoid, one celled and non-guttulate. They measure  $5.0-6.7 \times 1.7-3.0\mu$ ; conidia when placed in water swell considerably and

become bi-cellular before they germinate. Germ tubes are developed from both ends.

Wheat seedlings and ears inoculated with this fungus gave positive results. Brownish to blackish elongated lesions were formed on the stem ; on the glumes typical diffused elongated tobacco coloured lesions develop ; on the kernels enclosed by the inoculated glumes the embryo end was discoloured and at times there were also lesions on the pericarp.

#### TAXONOMY

Our fungus belongs to the family *Phomaceae*, sub-family *Hyalosporae*. In this sub-family, it is very near the genus *Phoma* Fr., em Desm. According to Saccardo [1884] in *Phoma* the pycnidia are not beaked, the ostiole is minute or obsolete, and the spores are mostly two-guttulate. As our fungus has not these characteristics it is doubtful if it can be placed in this genus. The important difference between this genus and *Pseudophoma* v. Hoehn., according to Clements and Shear [1931], is that the latter has rostrate pycnidia and the spores are hystogenic. Von Hoehnel [1916] gives the following description of the new genus created by him :—‘Stromata sub-epidermal ganz pycnidenahnlich, mit allseitig gleichmassig entwickelter, gut abgegrenzter Kruste, oben mit schnabelartigem Fortsatz, der (allein) nach aussen durchbricht. Conidien je eine aus einer Gewebszelle des Stromainnern histolytisch entstehend, zylindrisch-stabchenartig, zeimlich gross, durch den schliesslich oben ausbrockelnden Schnabelfortsatz entleert.’\* On wheat glumes and kernels thick walled bodies, brown to black in colour are developed in the tissues of the host, completely or partially filling the thickness of the glume or the pericarp ; whether in some cases at least, these pycnidial bodies are ‘stromata ganz pycnidenahnlich’, it is difficult to say ; but in cultures, on agar media wheat stems and bran and on moist filter papers, there is no stromatic development ; pear shaped or globular thick-walled pycnidia with a short but distinct beak are developed, singly or in clusters ; the beak is clearly ostiolate. The conidia do not seem to be developed by hystolysis ; the basidia are obsolete.

As our fungus has rostrate pycnidia with a distinct ostiole it is provisionally placed in the genus *Pseudophoma* v. Hoehn., even though it does not wholly answer to the description given by v. Hoehnel.

#### *Pseudophoma* sp.

Pycnidia sub-epidermal, without stromata, thick-walled, coriaceous to carbonous, pear shaped or sub-globose, with a short beak, ostiolate  $38-53 \times 152-228\mu$  ; basidia obsolete ; conidia hyaline, elliptical or ovoid one-celled, non-guttulate, escaping through the ostiole in long tendril,  $5.0-6.7 \times 1.7-3.0\mu$  ; germination bi-polar ; when placed in water conidia swell and become bi-cellular.

#### *Nigrospora sphaerica* (Sacc.) Mason

A *Nigrospora* was isolated both from the ‘black point’ affected wheat kernels and from spotted glumes of rice (*Oryza sativa*).

\*My thanks are due to Dr G. W. Padwick, Imperial Mycologist, and Dr B. B. Mundkur, Assistant Imperial Mycologist, Imperial Agricultural Research Institute, New Delhi, for very kindly supplying me the original descriptions of *Phoma* and *Pseudophoma*.

The growth of the fungus on wheat kernels incubated on moist filter papers under aseptic conditions is white and sparse ; later the mycelium is chiefly appressed to the surface of the kernel and the filter paper ; it does not form a compact mass ; the white colour is soon replaced by a diffused black or brownish colour—a sort of pepper and salt colour—on account of the development of conidia bearing hyphae which are brown in colour. The conidia are borne singly on short swollen or vesicular basidia, which may be hyaline or brown in colour. The conidia are broadly elliptical with rounded ends or are sub-globular, when seen from the side ; they are round when seen from the apex ; they are deep, dark brown or black in colour and opaque ; under high magnification they are seen to have a central lighter coloured globular area ; the short diameter of the conidium is in continuation of the vertical axis of the basidium. The conidia borne on the mycelium originating from the incubated wheat kernels are smaller than those produced in cultures. The former measure  $13.0-19.0 \times 9.5-15.0\mu$  ; those developed on rice meal agar cultures measure  $15.0-26.0 \times 11.0-18.7\mu$ . The *Nigrospora* isolated from spotted rice glumes is similar to that isolated from wheat kernels.

Miyake [1910] has described *Epicoccum hyalopes* Miyake on rice glumes in Japan which to Mason [1927], from the description given, ' seems undoubtedly to be a *Nigrospora* '. As the conidia measure  $14-18 \times 13-15\mu$ , Mason considers it to be *N. sphaerica* (Sacc.) Mason. Palm [1918] has described *N. javanica* Palm from wheat glumes. The conidia measure  $22-30\mu$  ; they are much bigger than those developed by the fungus isolated from wheat and rice by us. This fungus is, therefore, considered to be *Nigrospora sphaerica* (Sacc.) Mason.

Inoculations of seedlings and ears of wheat and rice isolated from these two hosts have given negative results.

*Sclerotium rolfsii* Sacc.

Innumerable ' black point ' affected kernels have been incubated under aseptic conditions ; but this sclerotial fungus, was isolated only from half a dozen kernels.

This fungus was identical with that isolated from roots of wilted wheat plants and of other hosts such as tomatoes, potatoes etc. It is, therefore, not necessary to give a detailed description of the fungus.

Inoculations of wheat seedlings and green ears were successful.

*Rhizoctonia* sp.

This fungus has often been isolated from ' black point ' wheat kernels and from spotted glumes of rice (*Oryza sativum*).

On rice glumes at first a brownish speck is visible ; this increases in size elliptically ; ultimately the centre of the lesion turns white and the margins black or brownish black. The white or the central part of the lesion is dry and slightly depressed, it ultimately cracks ; minute black sclerotia-like bodies are visible even with the naked eye in the white part of the lesion. The lesion is confined only to the glume ; the rice grain or seed is normal.

The sclerotia are globular and black in colour ; they measure  $23-76 \times 23-61\mu$ .

Inoculations of wheat and rice seedlings and ears were unsuccessful.

## SUMMARY

An account of some fungi not previously recorded on 'black point' affected wheat kernels is given. The fungi described are *Cochliobolus tritici* n. sp., *Helminthosporium* sp. A. and H. sp. B., *Pseudophoma* sp., *Nigrospora sphaerica* (Sacc.) Mason and *Rhizoctonia* sp. *Sclerotium rolfsii* Sacc. has also been isolated from 'black point' affected kernels.

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## EXPLANATION OF PLATE XXIX

*Cochliobolus tritici* n. sp.

- FIG. 1. Section of a wheat pericarp through a perithecium  
 FIGS. 2 & 3. Perithecia with and without beaks  
 FIG. 4. Asci with paraphyses  
 FIG. 5. A group of asci  
 FIG. 6. A stipitate ascus  
 FIG. 7. A non-stipitate ascus  
 FIG. 8. Asci showing the helicoid arrangement of ascospores  
 FIG. 9. Ascospores

*Pseudophoma* sp.

- FIG. 10. Section of a wheat pericarp through pycnidia  
 FIG. 11. Section of a wheat glume through pycnidia  
 FIG. 12. Pycnidia with beaks

*Helminthosporium* sp. A

- FIG. 13. A group of conidia

# THE FIXATION OF ELEMENTARY NITROGEN BY SOME OF THE COMMONEST BLUE-GREEN ALGAE FROM THE PADDY FIELD SOILS OF THE UNITED PROVINCES AND BIHAR

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## INTRODUCTION

AT the present time, evidence has been adduced to show that one of the probable causes for the preservation of the fertility of the paddy field soils is through the fixation of atmospheric nitrogen. In this connection Sen [1929] has demonstrated the presence of a nitrogen-fixing bacterium within the root of the rice plant, after the manner of the Leguminosae, while Viswanath [1932] has obtained indications that the rice plant itself possesses the power of assimilating elementary nitrogen. De [1936], working with a mixed culture of algae and bacteria, came to the conclusion that the fixation of nitrogen in rice soils under water-logged conditions is an algal process, while the absence of fixation in the cultures kept in the dark implies that bacteria cannot alone be involved. De and Bose [1938] found that in the water-logged period conditions are unfavourable for certain bacteria like *Azotobacter* which are unlikely to be very active at this time. By far the most important works on this subject have been those of Fritsch and De [1938] and De [1939]. They have concluded that nitrogen-fixation in these soils is purely through the agencies of algae and the part played by bacteria is relatively unimportant and possibly nil. They further found that nitrogen-fixation was confined to species of *Anabaena*, while *Phormidium foveolarum* afforded no evidence of fixation. Lately Uppal, Patel and Daji [1939] have shown that *Azotobacter* plays an important rôle in the nitrogen recuperation of rice soils at Karjat.

The present work arose out of the observation that in the paddy field soils of the United Provinces and Bihar there is an universal growth of a plant community constituted mainly by *Aulosira fertilissima* Ghose intermingled with filaments of *Anabaena ambigua* Rao, *Anabaena fertilissima* Rao and *Cylindrospermum gorakhporensis* Singh. This association forms a thick and compact stratum, and sometimes it becomes so extensive as to cover the surface of a field completely, interrupted only at places where paddy plants grow out. At the close of the harvest period the above blue-greens were observed to be reproducing freely by spores. After a fortnight or so it was found that the plants disintegrated and died, leaving behind only the spores for perennation. The presence of a large number of spores in the

upper layers of the soil was avoided of, and it was thought desirable to start cultures with these spores because of the little chances of contamination from bacteria and fungi.

For the sake of comparison *Protosiphon botryoides* (Kütz.) Klebs forma *parieticola* Iyengar, a member of the Chlorophyceae, was isolated from a paddy field of the Benares district. It was observed that the vesicles of the alga contained a number of thick-walled cysts, some of which were also liberated in the soil. After a week the plants died leaving behind only the cysts. The cultures in the present case were, therefore, started with the cysts.

#### CULTURE METHODS AND ISOLATION OF THE ALGAE

The culture solution used for the growth of these organisms was De's modification [1939] of Benecke's solution [cf. Kufferath, 1930] substituting  $\text{KNO}_3$  for  $\text{NH}_4\text{NO}_3$ . Its composition is as follows:  $\text{KNO}_3$ , 0.2 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 gm.;  $\text{CaCl}_2$ , 0.1 gm.;  $\text{KH}_2\text{PO}_4$ , 0.2 gm.;  $\text{FeCl}_3$ , (1 per cent), 2 drops; water (pyrex distilled), 1,000 c.c. In some experiments sterilized soil-extract was used as the basal medium; in others where the effect of changes in pH of the culture medium on growth of the algae and their nitrogen-fixing capacity was to be studied the above modification of Benecke's solution was buffered with potassium phosphates (mono-, di-, or tri-phosphates) to give the desired pH; while still in others where the importance of K and Ca ions on growth and activity of these plants was investigated various other modifications were used. In some cases nitrogen-free media were utilized and those generally used were: (1) modified Benecke's solution with the omission of  $\text{KNO}_3$ , (2) solution containing,  $\text{K}_2\text{HPO}_4$ , 0.5 gm.;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 gm.;  $\text{CaSO}_4$ , 0.1 gm.;  $\text{FePO}_4$ , 0.1 gm.;  $\text{Ca}_3(\text{PO}_4)_2$ , 1.0 gm.;  $\text{FeCl}_3$  (1 per cent), 2 drops and water (pyrex distilled), 1,000 c.c.

In order to obtain uni-algal cultures the following method was adopted:—Soil blocks without the least disturbance of the surface layers were brought to the laboratory and examined under a dissecting microscope. This revealed a large number of spores mostly on the surface of the soils. Also, the spores of the different species of algae under consideration were found in groups, thereby facilitating a good deal of their isolation, which was carried out as follows: The lumps of spores were removed from the soil with a pair of hot needles on a clean sterilized glass slide in a drop of sterilized water and under the microscope the adhering soil particles were, as far as possible, removed. Next, the spores were transferred with hot forceps to a test tube containing a little sterilized distilled water and closed with a rubber stopper and shaken vigorously. The suspension was then allowed to stand for 15 minutes and the supernatant turbid liquid was decanted off. This process was repeated several times until the supernatant liquid became perfectly clear. Finally the spores and in the case of *Protosiphon botryoides* the cysts along with a few c.c. of the liquid were transferred to a sterilized centrifuging tube in sterilized distilled water and centrifuged. The suspension was further diluted five times and again centrifuged. A loopful of this suspension was pipetted out by means of a hot pipette and transferred to several agar plates, and these were then exposed to light when after 15 or 20 days many showed good growth. Numerous filaments radiated from the points of inoculation, and single healthy

ones were selected and their positions marked with Indian ink under a microscope. Portions of the agar including such marked areas were then cut out, and transferred to the liquid medium mentioned previously and allowed to grow in 250 c.c. pyrex Erlenmeyer flasks.

For getting bacteria-free cultures silica gel plates were utilized. The silica gel was prepared by mixing equal volumes of hydrochloric acid (sp. gr. 1.1) and potassium silicate solution (sp. gr. 1.06). Merck's sodium silicate pure crystals were used and the solution was made up with cold water. In a number of 9 cm. Petri dishes, 40 c.c. portions of the mixture were placed and after 48 hours when the gel had hardened, the plates were first washed in running tap water until free from acid and subsequently several times with boiled distilled water. Each plate was then impregnated with 5 c.c. of the following solution:  $\text{KNO}_3$ , 0.1 gm.;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 gm.;  $\text{K}_2\text{HPO}_4$ , 0.2 gm.;  $\text{CaCl}_2$ , 0.1 gm.;  $\text{FeCl}_3$  (1 per cent), 1 drop; water (pyrex distilled), 100 c.c. Finally the plates were exposed to a temperature of 60° C. until the surface of the gel was fairly dry and then sterilized in an autoclave at a pressure of one and a half atmosphere for 20 minutes.

A loopful of the centrifuged suspension containing at the most five spores or cysts on the average was pipetted out and transferred by means of a hot sterilized pipette to the centre of a sterilized silica gel plate and spread over the surface by means of a hot glass rod. After this the plates were exposed to diffused light obtained from a north window in the laboratory. It took about a month or so for the spores to germinate and form algal filaments and in the case of the cysts the time taken was a little longer. After the appearance of the filaments a little of each was transferred to one of the soil-extract-nitrate-cane-sugar-agar plates described below, and, if found contaminated, fresh sub-cultures on silica gel were made as above and the process repeated. When one or more colonies were obtained, which appeared to be pure when tested by the above method, they were transferred to a culture medium and allowed to grow for sometime. They were finally tested for purity by the methods described below. In this way *Aulosira fertilissima*, *Cylindrospermum gorakhporensense*, *Anabaena ambigua*, *A. fertilissima* and *Protosiphon botryoides* forma *parieticola* were obtained in pure cultures.

The following media, both solid and liquid, were used for testing the purity of the cultures: (1) nutrient agar, (2) soil-extract-nitrate-cane-sugar-agar (De's modification of Benecke's solution given above with 50 c.c. soil-extract and 15 gm. cane-sugar), (3) Beijerinck's medium containing 20 gm. mannite, 0.2 gm.  $\text{K}_2\text{HPO}_4$ , and 1,000 c.c. tap water, (4) medium containing 2 per cent mannitol, 0.2 gm.  $\text{K}_2\text{HPO}_4$  in 1,000 c.c., 10 gm.  $\text{CaCO}_3$  in 1,000 c.c. and 1,000 c.c. tap water. Several drops of a suspension of the supposedly pure algal growth were inoculated into the above media, which were then incubated for a week in the dark at 25° C., the presence or absence of turbidity and growth of bacterial colonies along the line of inoculation in liquid and solid media respectively, being taken as an index of the presence or absence of bacteria.

The isolation from bacteria by growing the algae on sterilized silica gel plates was also started with the uni-algal filaments but it was observed that some of the cultures produced turbidity when grown in the testing media.

It is, therefore, concluded that it is safer to start with spores in order to obtain pure cultures.

Nitrogen analyses were made by the macro-Kjeldahl method. At the end of an experiment the entire contents of the culture vessel (medium + alga) were poured into a Kjeldahl flask, any growth adhering to the side of the vessel being washed out with pure conc. sulphuric acid. Total nitrogen was estimated by the Gunning-Hibbard modification of Kjeldahl's method [Wright, 1939]. Digestion and distillation were carried out in the usual way, the ammonia evolved being absorbed in  $N/15$   $H_2SO_4$  and estimated by back titration with  $N/15$   $NaOH$ , using methyl red as an indicator. For the determination of small amounts of nitrogen,  $N/50$  acid and alkali were used and the acid boiled to drive off  $CO_2$  before titration.

Pyrex Erlenmeyer flasks of 250 c.c. capacity were used for growing the cultures and the mouth of each flask was plugged with cotton-wool. A dozen empty flasks were, at one time, sterilized in an autoclave at a pressure of one and a half atmosphere for 20 minutes. After 24 hours the flasks were subjected to a second heating under the same pressure to ensure the complete elimination of bacteria. Next in each one of these flasks 100 c.c. of the culture medium was kept, the plugs replaced, and the whole again heated twice after the manner described above. The flasks were then allowed to stand for two days before being inoculated with a suspension of the alga in water. All cultures were grown under laboratory conditions of temperature and pressure. An overhead Philips electric lamp of 250 watts provided illumination of constant intensity adjusted to such a height that it did not affect the temperature of the surroundings of the cultures. On the average the cultures were illuminated for 10 hours daily.

#### NITROGEN-FIXATION BY THE ALGAE

Five replicates were made for each treatment and the experiments were conducted in two series. The mean of the replicates and ultimately the mean of the series has been taken as the criterion of the nitrogen-fixing capacity of the algae in question. The results are presented in Tables I-IV.

1. *Aulosira fertilissima* Ghose. In both the series in all cases the growth of the alga in the beginning was quite rapid and it formed a membranous blue-green stratum on the surface of the culture medium, which in most of the flasks extended on the sides above the level of the liquid, irrespective of the medium containing nitrate, or not. After a week's incubation the algal stratum, in cultures with nitrate in the medium and especially in those having soil-extract as the basal medium, began to fade out and finally became pale or almost colourless; while in cultures without nitrate, it continued to retain its bluish-green tinge even after the second week. At the close of the second week, however, in some of the flasks, with nitrate and with nitrate and soil-extract, patches of green colour began to appear on the sides signifying thereby the renewed growth of the alga. The algal stratum, however, never became so thick and compact as that found in nature. After the third week's incubation the behaviour of the cultures as described above remained similar and it continued to be so even after the close of the experiment.

TABLE I

*Nitrogen-fixation by Aulosira fertilissima Ghose*

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Media	First series				Second series			
	Initial N	Final N	N fixed	Mean of replicate	Final N	N fixed	Mean of replicate	Mean of series
1. N-free (De's modification of Be-necke's solution without KNO <sub>3</sub> )	...	7.3	7.3	7.4	7.7	7.7	7.6	7.5
		7.6	7.6		7.4	7.4		
		7.2	7.2		7.6	7.6		
		7.4	7.4		7.5	7.5		
		7.5	7.5		7.8	7.8		
2. N-free + soil-extract	0.4	8.4	8.0	8.08	8.6	8.2	8.02	8.05
		8.5	8.1		8.3	7.9		
		8.5	8.1		8.5	8.1		
		8.6	8.2		8.3	7.9		
		8.4	8.0		8.4	8.0		
3. N-free + nitrate	2.9	5.8	2.9	2.92	6.0	3.1	2.68	2.8
		6.2	3.3		5.5	2.6		
		5.6	2.7		5.3	2.4		
		5.9	3.0		5.7	2.8		
		5.6	2.7		5.4	2.5		
4. N-free + nitrate + soil-extract	3.8	11.8	8.6	8.8	11.7	8.4	8.6	8.7
		12.2	8.9		11.6	8.3		
		12.4	9.1		12.0	8.7		
		12.0	8.7		11.9	8.6		
		12.1	8.8		12.3	9.0		

2. *Cylindrospermum gorakhporens* Singh. The growth of *Cylindrospermum gorakhporens* was always submerged and it formed a thick dull-green irregular stratum on the bottom of the flask. In soil-extract-nitrate medium the growth was slow in the beginning and it was not until the commencement of the third week that the algal stratum became visible at the bottom of the flask. In N-free-soil-extract and N-free-nitrate media, however, the growth was quite rapid at the start but after a week's incubation the stratum became pale and finally colourless. It renewed growth after the second week's incubation. On the whole, in the latter two media, the algal cells were abnormally developed. The heterocysts were very much elongated and the cell-contents, which were at first granular became homogenous and pale yellow.

3. *Anabaena ambigua* Rao. The growth in case of this alga was, in the beginning, submerged, the colonies being in the form of narrow cylinders, standing almost erect. Later, however, they had the tendency of rounding

up and coming up on the surface of the culture medium. In the N-free-soil-extract and N-free-nitrate media the growth was quick at the start ; but after two weeks' incubation the colonies began to lose colour and became diffuent, finally getting mixed up with the medium. In the soil-extract-nitrate medium, however, the algal colonies were quite intact till the close of the experiment.

TABLE II

*Nitrogen-fixation by Cylandrospermum gorakhporensense Singh*

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Media	First series				Second series			
	Initial N	Final N	N fixed	Mean of replicate	Final N	N fixed	Mean of replicate	Mean of series
1. N-free (De's modification of Benecke's solution without KNO <sub>3</sub> )	...	4.5	4.5	4.28	4.1	4.1	4.02	4.15
		4.2	4.2		3.8	3.8		
		3.8	3.8		3.9	3.9		
		4.6	4.6		4.2	4.2		
		4.3	4.3		4.1	4.1		
2. N-free + soil-extract	0.4	4.8	4.4	4.68	5.4	5.0	4.78	4.78
		5.2	4.8		5.5	5.1		
		5.3	4.9		4.9	4.5		
		5.2	4.8		4.9	4.5		
		4.9	4.5		5.2	4.8		
3. N-free + nitrate	2.9	4.9	2.0	2.46	4.8	1.9	2.3	2.38
		5.5	2.6		4.7	1.8		
		5.6	2.7		5.4	2.5		
		5.4	2.5		5.6	2.7		
		5.4	2.5		5.5	2.6		
4. N-free + nitrate + soil-extract	3.3	8.3	5.0	4.82	8.6	5.3	5.0	4.91
		7.9	4.6		8.3	5.0		
		7.8	4.5		8.2	4.9		
		8.2	4.9		8.0	4.7		
		8.4	5.1		8.4	5.1		

4. *Anabaena fertilissima* Rao. The growth, in this case, consisted of small spherical colonies of blue-green colour at the bottom of the flask. These after a week's incubation aggregated to form irregular bigger colonies and the colour changed to brownish-black. In the N-free soil-extract and N-free nitrate media the growth of the alga was quite quick and it remained so till the close of the experiment. In the N-free and N-free nitrate-soil-extract media the growth was slow in the beginning but after the third week's incubation it became quite vigorous,

TABLE III

*Nitrogen-fixation by Anabaena ambigua Rao*

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Media	First series				Second series			
	Initial N	Final N	N fixed	Mean of replicate	Final N	N fixed	Mean of replicate	Mean of series
1. N-free (De's modification of Bencke's solution without $\text{KNO}_3$ )	...	3.8	3.8	3.6	3.5	3.5	3.56	3.58
		3.6	3.6		3.4	3.4		
		3.3	3.3		3.7	3.7		
		3.7	3.7		3.6	3.6		
		3.6	3.6		3.6	3.6		
2. N-free + soil-extract	0.4	4.2	3.8	4.06	4.7	4.3	4.22	4.14
		4.6	4.2		4.5	4.1		
		4.5	4.1		4.6	4.2		
		4.6	4.2		4.6	4.2		
		4.4	4.0		4.7	4.3		
3. N-free + nitrate	2.9	5.0	2.1	2.26	5.3	2.4	2.3	2.28
		5.4	2.5		5.5	2.6		
		4.8	1.9		5.4	2.5		
		5.2	2.3		4.9	2.0		
		5.4	2.5		4.9	2.0		
4. N-free + nitrate + soil-extract	3.3	9.3	6.0	5.58	8.6	5.3	5.66	5.62
		9.2	5.9		8.9	5.6		
		8.6	5.3		8.7	5.4		
		8.7	5.4		9.4	6.1		
		8.6	5.3		9.2	5.9		

5. *Protosiphon Botryoides* (Kütz.) Klebs forma *parieticola* Iyeng. The growth of the present alga was completely retarded in N-free and N-free-soil-extract media. There was slight growth in the beginning in the N-free-nitrate medium but after the third week's incubation a few vesicles of the plant were found attached to the sides of the flask above the culture medium. In N-free-nitrate-soil-extract medium, however, the growth was plentiful, and clusters of dark-green vesicles appeared at the bottom and the sides of the flask. There was, however, no increase in the nitrogen content, which meant that the alga was unable to fix nitrogen from the atmosphere. After the fourth week's incubation in the last medium the vesicles began to disintegrate fast and within four days the plant died completely, perhaps due to the deficiency in the nitrogen content of the medium. The same observation was recorded in regard to the N-free-nitrate medium, as the growth was completely inhibited after the fourth week's incubation.

TABLE IV

*Nitrogen-fixation by Anabaena fertilissima Rao*

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Media	First series				Second series			
	Initial N	Final N	N fixed	Mean of replicate	Final N	N fixed	Mean of replicate	Mean of series
1. N-free (De's modification of Bennecke's solution without KNO <sub>3</sub> )	...	4.6	4.6	4.6	4.7	4.7	4.64	4.62
		4.8	4.8		4.9	4.9		
		4.5	4.5		4.5	4.5		
		4.6	4.6		4.4	4.4		
		4.5	4.5		4.7	4.7		
2. N-free + soil-extract	0.4	5.7	5.3	5.3	5.5	5.1	5.14	5.22
		5.6	5.2		5.4	5.0		
		5.7	5.3		5.6	5.2		
		5.7	5.3		5.6	5.2		
		5.8	5.4		5.6	5.2		
3. N-free + nitrate	2.9	5.8	2.9	2.76	5.3	2.4	2.74	2.75
		5.9	3.0		5.8	2.9		
		5.3	2.4		5.7	2.8		
		5.6	2.7		5.6	2.7		
		5.7	2.8		5.8	2.9		
4. N-free + nitrate + soil extract	3.3	9.8	6.5	6.56	10.2	6.9	6.66	6.61
		10.0	6.7		10.0	6.7		
		9.6	6.3		10.0	6.7		
		9.7	6.4		9.8	6.5		
		10.2	6.9		9.8	6.5		

## FACTORS DETERMINING GROWTH AND NITROGEN-FIXATION

1. *Illumination.* The optimum light intensity was found to depend to a marked extent upon the growth conditions, particularly, the medium used. In nitrogen-free media the growth and the nitrogen-fixation capacity of *Aulosira fertilissima* Ghose were accelerated to a marked extent with increasing light intensity but the cultures could not stand the direct sunlight of very high intensity for a long time as after the fifth week's incubation the algal cells began to disintegrate and finally disappeared completely. In diffused light, obtained from a north window, the growth proceeded slowly and it was not till the end of the third week's incubation that the algal stratum became visible. Later, however, the growth became greatly increased and the vigour of the cultures remained more or less constant till the close of the experiment. The nitrogen-fixation capacity also increased. These cultures were illuminated, on the average, for 10 hours daily. Some cultures were kept in the dark, and in these cases the growth appeared to be very slow and it remained so till the end of the experiment. The nitrogen-fixing

capacity of the alga also remained almost constant. But, it increased very considerably in such cultures as were provided with 1 gm. of sugar per 100 c.c. of the medium. It was, however, found that the best light conditions for maximum growth and nitrogen-fixation was intermittent light, i.e., when the cultures were daily kept alternately in diffused light and direct light for five hours. In media containing nitrogen the growth was greatest in direct light, but the nitrogen-fixation capacity of the alga was slightly retarded. The reason for the latter behaviour is obvious. The results of the above mentioned experiments, carried out with *Aulosira fertilissima* Ghose, are given in Table V.

TABLE V  
*Growth and nitrogen-fixation by Aulosira fertilissima Ghose under varying light conditions*

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Illumination		Medium used	Growth	N fixed
Daily period in hours	Light source			
10	Direct sunlight	N-free . . . . .	Vigorous, retarded totally after five weeks	6.3
10	Direct sunlight	N-free + soil-extract . . . . .	Vigorous, retarded totally after five weeks	6.8
10	Direct sunlight	N-free + nitrate . . . . .	Best . . . . .	4.2
10	Direct sunlight	N-free + soil-extract + nitrate	Best . . . . .	5.3
10	Diffused light	N-free . . . . .	Slow in the beginning, increased after third week and remained constant	7.8
10	Diffused light	N-free + soil-extract . . . . .	Comparatively quick . . . . .	6.6
10	Diffused light	N-free + nitrate . . . . .	Quick in the beginning, but after second week it was retarded	2.5
10	Diffused light	N-free + nitrate + soil-extract	Quick in the beginning, but after second week it was retarded	3.8
10	Dark . . . . .	N-free . . . . .	Very slow but remained constant	3.5
10	Dark . . . . .	N-free + 1 gm. sugar per 100 c.c. of medium	Very slow but remained constant	4.5
10	Dark . . . . .	N-free + soil-extract + nitrate	Very slow and did not continue long	2.3
10	Direct and diffuse light (five hours each)	N-free . . . . .	Excellent . . . . .	8.6
10	Direct and diffuse light (five hours each)	N-free + soil-extract . . . . .	Excellent . . . . .	7.6
10	Direct and diffuse light (five hours each)	N-free + nitrate . . . . .	Slow in the beginning . . . . .	4.7
10	Direct and diffuse light (five hours each)	N-free + nitrate + soil-extract	Slow in the beginning . . . . .	5.3

2. *Hydrogen-ion concentration.* The effect of pH on growth and nitrogen-fixation capacity was studied on *Aulosira fertilissima* Ghose. The results of the various experiments are given in Table VI. It is seen from this

table that a neutral or slightly alkaline medium is decidedly preferable for the alga. Growth could not take place below pH 6.5. It was initiated at pH 6.5 and with increasing pH it became more and more vigorous, the normal being realized at 7.2. At higher pH values, although the growth, to all outward appearances, was vigorous the algal cells were found to be abnormally elongated, especially the heterocysts. The nitrogen-fixation capacity of the alga also increased with increasing pH. It has also been observed that with longer period of incubation the pH of the medium begins to decrease, after three weeks' incubation in case of the nitrogen-free media and after a week's incubation in media containing nitrogen. This is perhaps due to the disintegration of the algal cells.

TABLE VI

*Growth and nitrogen-fixation at different pH by Aulosira fertilissima Ghose*  
(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Initial pH	Relative growth (after 20 days incubation)	N fixed	Final pH
5.2 . . .	None . . . . .	..	5.0
5.5 . . .	None . . . . .	..	5.2
5.7 . . .	None . . . . .	..	5.8
6.0 . . .	None . . . . .	..	5.4
6.3 . . .	None . . . . .	..	6.1
6.5 . . .	Slight . . . . .	2.6	6.0
6.8 . . .	Fair . . . . .	4.2	6.2
7.2 . . .	Normal . . . . .	7.8	6.8
7.4 . . .	Vigorous . . . . .	7.8	7.0
7.6 . . .	Vigorous . . . . .	8.1	7.3
8.0 . . .	Abnormal (decaying) . . .	6.5	7.5
8.4 . . .	Abnormal (decaying) . . .	5.8	7.3
8.8 . . .	Abnormal (decaying) . . .	2.5	7.8

3. *Calcium and potassium ions.* It was reported by Allison, Hoover and Morris [1937] that neither calcium nor strontium, at least in concentrations greater than traces, was necessary for growth in the presence of combined nitrogen for *Nostoc muscorum* Ag. In nitrogen-free medium, nitrogen fixation, however, decreases greatly in the absence of these ions, suggesting thereby

that they play a catalytic role in nitrogen-fixation, as in the case of *Azotobacter*. Similar experiments were conducted with *Aulosira fertilissima* Ghose. The results of these experiments are given in Table VII. These results are in close agreement with those of the above mentioned workers in so far as the behaviour of the calcium ion is concerned. It has also been observed that calcium carbonate as against calcium sulphate or calcium chloride is most effective for growth and nitrogen-fixation. In another series of experiments, where basal medium consisted only of  $K_2HPO_4$  and the inoculating culture was grown on a calcium-free medium, practically no growth was obtained, except where either combined nitrogen or calcium sulphate was added. Under such conditions the nitrogen-fixation capacity of the alga also increased.

TABLE VII

*Effect of Ca and K ions on growth and nitrogen-fixation in Aulosina fertilissima Ghose*

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days. Basal medium:  $K_2HPO_4$ , 0.75 gm.;  $MgSO_4 \cdot 7H_2O$ , 0.2 gm.; NaCl, 0.2 gm.  $FeCl_3 \cdot 6H_2O$ , 0.005 gm.;  $H_2O$ , 1,000 c.c.)

Treatment	Relative growth (after 20 days incubation)	N fixed
Control (basal medium)	None	
$CaSO_4$ , 10 mg.	Slight	3.2
$CaCl_2$ , 10 mg.	Slight	1.8
$CaCO_3$ , 10 mg.	Normal	8.2
$KNO_3$ , 10 mg.	Slight	2.3
$KNO_3$ , 10 mg. + $CaSO_4$ , 10 mg.	Normal	6.8
$KNO_3$ , 10 mg. + $CaCl_2$ , 10 mg.	Normal	5.7
$KNO_3$ , 10 mg. + $CaCO_3$ , 10 mg.	Vigorous	8.6

### DISCUSSIONS

A summary of our knowledge of the occurrence and rôle of blue-green algae in nature would seem to indicate that these organisms are of considerable importance in the maintenance of soil fertility [cf. Bharadwaja, 1940 and Stokes, 1941]. The results obtained by Allison and coworkers [1937] with the most active nitrogen-fixing blue-green alga, *Nostoc muscorum* Ag., as they maintain it, lend further support to the same viewpoint. A similar conclusion was reached by Bristol [1920], even though nitrogen-fixing ability of certain *Cyanophyceae* had not been demonstrated conclusively at that time. Petersen [1935], however, is doubtful about the economic importance of algae in soil, basing his views largely on the supposition that they make little growth except at the soil surface. He considers that the *Myxophyceae*

with the exception of *Nostoc punctiforme* and possibly a few others, cannot grow heterotrophically or fix nitrogen in the dark. Author's results with another active nitrogen-fixing blue-green alga, *Aulosira fertilissima* Ghose, isolated from the paddy field soils of the United Provinces and Bihar together with the recent results of Allison and coworkers [1937] with *Nostoc muscorum* Ag. and that of Winter [1935], as quoted by Allison and coworkers [1937] with *Nostoc punctiforme*, definitely contradict these two ideas which serve in large part as a basis for Petersen's viewpoint. Whether *Aulosira fertilissima* Ghose actually makes an appreciable growth in soil where light does not penetrate still remains to be determined, but it is at least of interest to know that it has the capacity to do so. Moreover it has been observed that *Aulosira fertilissima* not only makes an appreciable growth in the dark but its nitrogen-fixing capacity also remains fairly considerable.

If the results reported here in regard to *Aulosira fertilissima* Ghose are proved to be typical, it would seem that the nitrogen-fixing blue-greens thrive best in nearly neutral or slightly alkaline soils, preferably partly shaded and where moisture is abundant. *Aulosira fertilissima* also shows abundant growth in freshwaters, where the pH is very low, and sometimes in distinctly acidic soils. These studies suggest that even in acid soils it may be able to continue to multiply at the surface, because by growing together and constantly removing carbon dioxide from the soil during photosynthesis it may increase the pH locally. It has also been observed that it has the tendency to reduce the pH of the medium during its growth, probably due to the liberation of organic acidic substances during the death and decay of certain of its cells. In short it can be said that the alga has a great buffering capacity. Its gelatinous sheath also enables the organism to withstand remarkably dry soil conditions, as Fritsch [1932, 1936] and others have pointed out with *Nostoc* and other blue-greens.

The nitrogen-fixing algae, growing near the soil surface, are unique in being able to obtain both their carbon and nitrogen from the air. This, of course, explains why they appear so soon on new volcanic soils and in other places where the soil is too poor to support most other forms of plant life.

The results of the various experiments embodied in the text bring out two important conclusions: (1) blue-green algae, apart from the species of *Nostoc* and *Anabaena*, in pure cultures free from bacteria and other micro-organisms, are able to utilize and fix nitrogen, (2) the green algae appear to take no part in the fixation process, though the observations have been limited and confined to only *Protosiphon botryoides* (Kütz.) Klebs forma *parieticola* Iyeng. So far as the first one is concerned, it has been observed that the results of carefully controlled experiments, on a comparative basis, have shown that *Aulosira fertilissima* Ghose, a very prominent participant in the algal flora of the paddy fields of the United Provinces and Bihar, fixes the greatest amount of nitrogen out of the other blue-greens under consideration. The results of another series of experiments show that nitrogen-fixation capacity of *Cylindrospermum gorakhporensense* Singh, another common blue-green alga from the same localities, is by no means insignificant. The other forms that were isolated from these soils are *Anabaena ambigua* Rao and *Anabaena fertilissima* Rao, which also appear to fix considerable amount of nitrogen

from the atmosphere. It is legitimate, therefore, to conclude from the above observations that the recuperation of nitrogen in the paddy field soils of India is an algal process, a view expressed also by De [1939]. It may be pointed out that the paddy field soils harbour a large number of algae [Singh, 1939 mostly blue-greens, that are likely to play an immense role in the economy of these soils. Besides their capacity of fixing atmospheric nitrogen they are beneficial in aerating the upper layers of the submerged soils [cf. Harrison and Aiyer, 1914].

Again, the present investigation has a bearing upon the theory put forward by Dhar and coworkers [1934-36], that nitrification in tropical soil is more photochemical than bacterial, and that nitrogen-fixation is a question of energy relations because more of nitrogen is fixed in soils mixed with energyn providing materials, such as carbohydrates, celluloses and fats in sunlight or artificial light than in the dark, although the *Azotobacter* numbers in the dark are very much greater than in the light. If this hypothesis is correct, then all chlorophyll-bearing plants and plant organs should fix atmospheric nitrogen. But, this is not so, as all attempts to find nitrogen-fixation by higher plants other than leguminous, since the classical work of Hellriegel and Wilfarth [1888], have failed. The green leaf is undoubtedly the prime source of energy on this planet, where a chain of complex chemical reactions involving energy changes and transference take place but no fixation of nitrogen. From the algal side we have the results of numerous investigations which definitely show that the green algae, which by no means are less in their energy relations to the blue-greens, are unable to fix nitrogen. Kossowitsch [1894], by isolating a grass-green alga, *Cystococcus*, in pure culture, found that it could not fix nitrogen. Schramm [1914] worked with seven species of the green algae and found that in the absence of combined nitrogen no growth took place, so he concluded that these algae could not under these conditions assimilate free nitrogen. Similar results were also obtained by Muenscher [1923] for *Chlorella*. Investigations so far done on nitrogen-fixation have alleged this function in only *Nostoc* and *Anabaena* species, though Copeland [1932] mentioned forms, such as *Oscillatoria princeps*, *Osc. formosa*, *Spirulina labyrinthiformis* and *Phormidium laminosum*. The present work has added two new forms possessing considerable nitrogen-fixing capacity to the existing list—*Aulosira fertilissima* Ghose and *Cylindrospermum gorakhporensense* Singh. The nitrogen-fixing capacity of these plants may perhaps be due to their peculiar metabolic activities, about which very little is yet known; and not to some simple energy relations which Dhar and his coworkers explain.

#### SUMMARY

The investigation deals with the nitrogen-fixation ability of some of the commonest blue-green algae—*Aulosira fertilissima* Ghose, *Cylindrospermum gorakhporensense* Singh, *Anabaena ambigua* Rao and *Anabaena fertilissima* Rao—isolated from the paddy field soils of the United Provinces and Bihar. It has been found that nitrogen recuperation in these soils is an algal process and the greatest fixation, amounting to 8.05 mg. per 100 c.c. of the N-free medium in 45 days, is obtained by *Aulosira fertilissima* Ghose.

For the sake of comparison, a grass-green alga, *Protosiphon botryoides* (Kütz.) Klebs forma *parieticola* Iyeng. was also isolated from a paddy field of Benares district and studied in the same way. It has been found that this alga does not fix nitrogen.

Factors, such as, illumination, pH, and the effect of Ca and K ions on growth and nitrogen-fixation ability of *Aulosira fertilissima* Ghose, have also been investigated.

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y. Bharadwaja, for his kind guidance and criticism throughout the course of this investigation.

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# THE COLD STORAGE OF FRUITS IN THE PUNJAB

## I. CITRUS FRUITS : MALTA (*CITRUS SINENSIS*) AND SANGTRA (*C. NOBILIS*)

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(With Plate XXX and four text-figures.)

**C**OLD storage is absolutely indispensable for the healthy development of fruit industry and it is no exaggeration to say that, but for the existence of cold storage facilities in other countries, their fruit industries would not have survived for any length of time. The main reason for this is that most of the fruits are so easily perishable that after removal from the trees, they cannot be stored even for a few days under ordinary atmospheric temperatures. Cold storage helps in extending the period of availability of fruits and thus reduces considerably the fluctuations in the prices of fruits. This enables the fruit growers to realize reasonable price for their fruits as they do not have to dump their produce in the market at any price. They can release the fruit from the cold storage as and when required to meet the demand of the market. Consumers, on the other hand, are able to secure fruits at reasonable price for a longer period in the market.

Although the fruit industry in this country has not, so far, made any great progress, still the profitable disposal of fruit has already become an acute problem for the growers. For instance, in the Punjab, Malta oranges in February can be had at almost Re. 1 to Rs. 2 per hundred, and Sangtra orange at even 12 annas a hundred, yet after two to three months these cannot be had at even five times the price of this. This is the condition in case of citrus fruits which have got a fairly good keeping quality. And worse is the situation in other fruits and vegetables which cannot be stored for even a day or two under ordinary atmospheric temperature prevailing in summer. When there is glut in the market, which is quite frequent, fruits and vegetables can be had at almost dirt cheap price. Tomatoes in June can be had even at eight annas a maund and the price easily goes up ten times after sometime.

Months of April to June are notorious in regard to the prevalence of many frightful diseases like typhoid when medical people in 99 per cent of the cases, do not recommend to patients anything else but the use of fresh fruit juices which are not available at this time of the year except those obtained from fruit stored in cold stores. In short, the cold storage can prove a boon to the growers and blessing to the consumers and it would be idle to expect sound development of fruit industry in the absence of cold storage enterprise.

The importance of this problem was realized long ago and the Fruit Specialist, Punjab, had submitted proposals several years back for the installation of a cold storage plant for experimental purposes but financial stringency always

stood in the way. Fortunately, a vigorous enterprise was started about four years back in the form of Cold Storage Company of Northern India and at their request the Imperial Council of Agricultural Research, in active cooperation of the Punjab Government, agreed to start experiments on the cold storage of fruits. The Cold Storage Company supplied a small plant of one and a half ton capacity, which was purchased later on by the Punjab Government. The Punjab Government and the Imperial Council of Agricultural Research agreed to share the other expenses of the cold storage scheme in equal proportions. As the plant had a very small capacity, only a few fruits could be experimented upon at a time. Citrus being the most important fruit of the province, naturally received special attention although some other fruits like mangoes, pears and grapes were also experimented upon on a small scale. This paper, however, deals with the cold storage trials of Malta and Sangtra oranges alone.

#### REVIEW OF LITERATURE

Cold storage of Malta orange has been a subject of thorough study in other countries like the United States of America, Australia and South Africa. The results obtained in different countries vary considerably. Young and Read [1930] working on Valencia and Navel oranges found that 38°F. and 45°F. respectively were suitable temperatures for these fruits and these varieties kept well for three to four months and 3½ months respectively at above temperatures. There was little change in sugar content of juice during cold storage. At 32°F. the fruit became bitter in taste after five weeks. Ramsey [1915]—cited by Ray Nelson [1933]—recommended the employment of temperatures considerably above 32°F., i.e. 38°-40°F. for oranges. Overholser [1930]—cited by Karmarkar [1941]—found that the temperatures of 36°-38°F. were most satisfactory. At higher temperatures the losses were heavy due to shrinkage and decay and lower temperatures caused pitting of the rind. Friend and Bach [1932] observed that at 44°-45°F., Valencia orange could be kept very satisfactorily for long periods. Wardlaw [1933] stated that 40°F. was well suited for the storage of certain classes of citrus fruits (except limes and grapefruit). He found that the loss in weight was largely a function of size and maturity and was directly related to the area of fruit surface exposed. He also advocated the use of cellophane or other thin strong wrappers, suitably impregnated with wax or other water proofing substances instead of ordinary wrappers. Stahl and Camp [1936] found that 37·5°F. proved to be the optimum temperature for the storage of unwrapped, untreated oranges and the temperatures below this were better than temperatures above. Stahl, Camp and Fifield [1936] also recommended that wrapping was better than none at all. Samisch [1936] working on the gas storage of Valencia oranges remarked that the fruits stored at 32°F. and 36°F. kept better and showed no wastage as compared to fruits stored at 45°F. and 70°F. Tomkins [1937] stated that 70 per cent relative humidity reduced wastage as compared to saturated atmosphere. If ventilation was sufficiently restricted to allow the accumulation of 10 per cent carbon dioxide, wastage might be increased. Cheema, Karmarkar and Joshi [1937] found 40°F. to be the best temperature for the storage of Nagpur oranges (mandarins) and that washing with antiseptic solution was of no particular

value in lengthening the storage life of the fruit. Cheema and others [1939] found that Malta oranges from the Punjab kept for four months at 40°F. in good condition without any wastage. Stahl and Cain [1937] recommended high humidity and a temperature of 37°F. with 6 per cent carbon dioxide plus 12 per cent oxygen, as the most suitable conditions for the storage of oranges. Tindale, and coworkers [1938] stated that 40-42°F. was most suitable temperature for the storage of Washington Navel oranges at which these kept in good condition for 12 weeks. Storage life of Valencia Late oranges at 40-42°F. was 14 weeks. Tomkins [1936] working on Jaffa oranges stated that as judged by the time taken for development of 10 per cent waste, storage at 41°F. was preferable to storage at 50°F. Early season (November) fruit was more susceptible to rotting by green mould than the late season (March and May) fruit. Fiddler and Tomkins [1938] found that dipping oranges in 2 per cent sodium hydroxide was as effective as 5 per cent borax and leads to less injury to skin. One per cent borax plus one per cent sodium hydroxide were as effective as 5 per cent borax alone. Vander Plank and others [1937, 1938] emphasized that the effect of temperature varied with the nature of fruit stored. The temperatures 50-55°F. were beneficial for the storage of under-coloured or greenish oranges. At these temperatures the fruit coloured well in storage and wastage was as low as at 39°F. The fruit stored at 50°F. for about two months was liable to become stale while at 39°F. the flavour was well maintained. Williams [1938] observed that the fruit stored in room at a temperature of 36-38°F. kept much better than that at lower or higher temperatures. Wrapped fruit was better than unwrapped fruit. Karmarkar and Joshi [1940] found that percentage loss in weight of small fruit was always greater than that of big fruit except in case of grape fruit at 68°F. Rose and others [1938] recommended the use of 32-34°F. and 80-90 per cent humidity for the storage of Washington Navel and Valencia Late oranges, at which storage life was 8-10 weeks.

#### MATERIAL

Two important citrus fruits, viz. Malta orange (*Citrus sinensis*) and Sangtra (*C. nobilis*) were included in the cold storage trials during 1938 and 1939.

*Malta oranges.*—Five varieties of Malta orange, viz. Common, Blood Red, Valencia Late and Seville were stored during 1938-39 and 1939-40. Musambi was tried in 1940-41. Malta Common, widely cultivated, is a heavy bearer, and normally quite pleasant in taste. Blood Red is the choicest variety of the Punjab and is liked very much due to the red colour of its flesh, pleasant taste, and agreeable aroma. Valencia Late is a late ripening variety, and possesses good flavour. Seville is a heavy bearer. Musambi is popular with invalids as it has very little acid.

Malta Common and Blood Red were obtained from S. Mangal Singh's Garden near Shahdara, Lahore, in the beginning of March; Valencia Late from the Indian Mildura Fruit Farms Ltd. Renala Khurd, in the second week of March and Seville was obtained from the Experimental Fruit Garden at Lyallpur in both the seasons in January. Musambi was obtained from Montgomery and Lyallpur districts during the second week of January.

**Sangtra oranges.**—Sangtra is most commonly cultivated in the plains, the fruit is puffy or loose skinned and is easily damaged. The fruit is a bit acidic (0.92 gm. citric acid per 100 c.c. juice) but when mature, is quite pleasant in taste. Two lots of Sangtra, one from Pathankote side and the other from Lyallpur, were tried during the two years of the investigations.

#### THE COLD STORAGE PLANT

The cold storage plant is designed for carrying out experiments on a small scale. The outer dimensions of the plant are  $14\frac{3}{4}$  ft.  $\times$   $7\frac{1}{2}$  ft.  $\times$   $7\frac{3}{4}$  ft. and has a storage capacity of one and a half tons. It consists of three small chambers designed to maintain three different temperatures (Plate XXX, fig. 1). The chambers are at present being cooled by cool air circulated by a fan over the cooling coils. Each chamber is divided into four compartments, and every compartment is fitted with three removable shelves. Individual chamber is served by an independent compressor-motor connected to a thermostatic switch. There is an 'air-lock'  $4\frac{1}{2}$  ft.  $\times$   $2\frac{1}{2}$  ft.  $\times$  7 ft. for each chamber.

Di-chloro-di-fluoro-methane commercially known as Freon or F-12, is used as refrigerant. It is non-inflammable and non-poisonous.

The plant was installed at the end of October, 1937. Since then many additions and alterations have been effected to get a closer and uniform range of temperature. Most of the changes have been in direction of electric installations done to get different speeds of circulating air. The diagrams of the electric installation in the beginning and at present are given in Fig. 1. With the present arrangements it is possible to control the air speeds, both when the compressor is working and when it is at rest. These controls are adjustable according to the requirements as to whether higher speed is required when the compressor is working or when it is at rest. This arrangement helps to minimize the fluctuations at the top and bottom of the chambers.

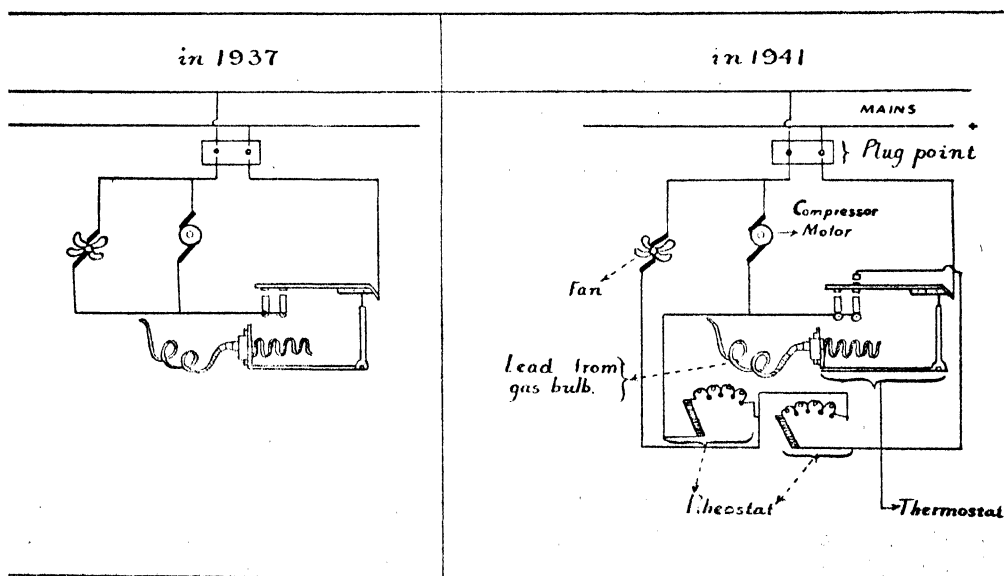


FIG. 1. Plan of electric installation in the cold storage plant

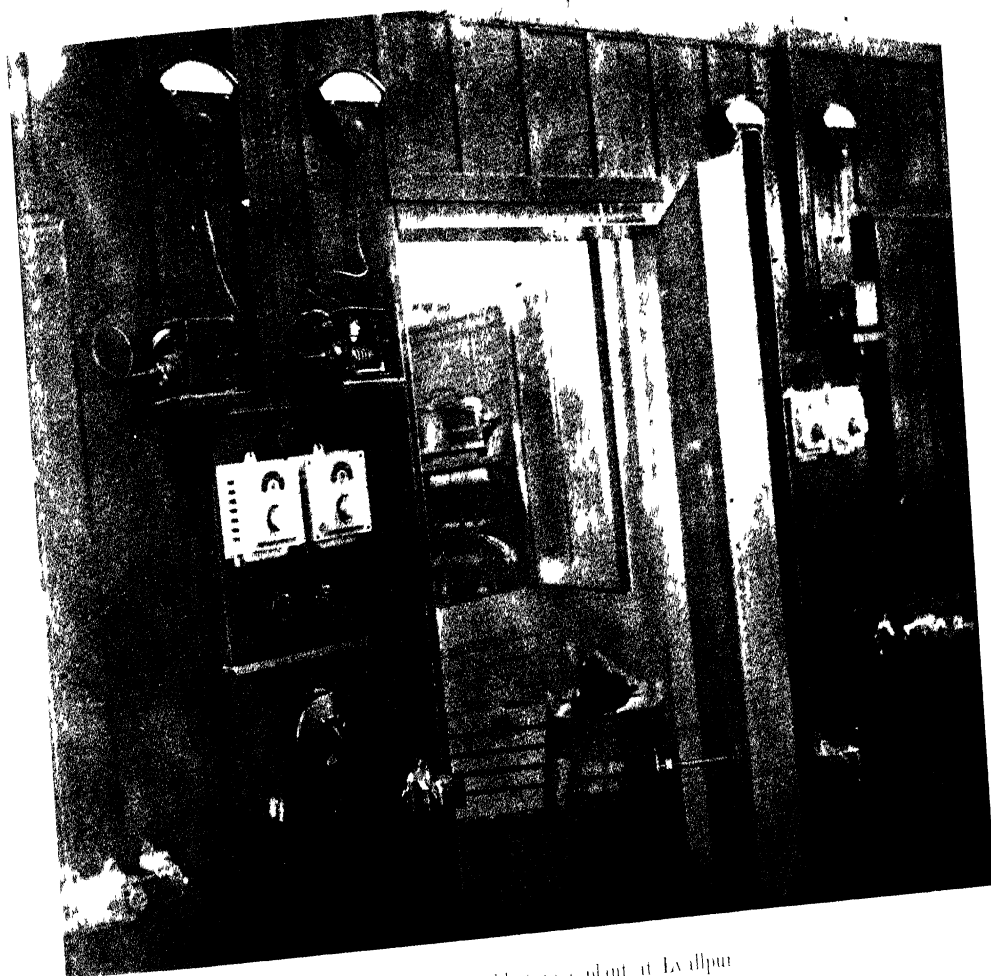


FIG. 1 Cold storage plant at Lxvillpur



FIG. 2 Chill-spot injury of fruit



### METHOD

The experiment was arranged to study the effect of size of fruit and different temperatures upon the storage life of different varieties of Malta and Sangtra oranges.

The fruit was graded into two sizes, viz. large and small. The diameter of large fruit varied from 7.2 cm. to 8.9 cm. and small fruit from 6.0 cm. to 7.2 cm. Half the number of fruits of each size was wrapped with butter-paper and the other half kept unwrapped. The fruit after wrapping was subjected to different ranges of storage temperature. The ranges of temperature in different chambers during the first trial were 24°-32° F., 36°-39° F. and 40°-44° F. But the fluctuations were reduced to a closer limit due to the provision of more piping and improvement in the air circulation, different temperatures ranged as 29°-32° F., 36°-39° F. and 40°-43° F.

The number of fruits used under each treatment (viz. three temperatures, two sizes and two wrappings) was 72 and the fruits were arranged in two rows in open trays. The trays were fitted with wooden splints at the base.

#### *Losses in weight of fruit*

For the purpose of determining the loss in weight during storage six additional fruits under each treatment were numbered and weighed individually at fortnightly intervals.

#### *Other physico-chemical analyses*

Observations in case of all the varieties stored were made on the general condition of the fruit after every two weeks. Percentage weights of peel and available juice were recorded as well as acid and sugar (total soluble solid) content of the juice determined [Trout *et al.* 1938] at four-week intervals. Four fruits were taken at random from each sub-lot at each occasion and the juice was extracted with the help of an electric driven 'Rose's cone' and then strained through a muslin piece with hand press.

*Storage life.* The fruit was considered properly stored so long as the wastage did not exceed 10 per cent.

*Keeping quality of fruits after removal from cold store.* Occasionally four fruits stored at each temperature were taken out of the cold store and placed at room temperature to study their keeping quality after removing from cold store.

*Study of rot organisms.* A study of rot organisms was made and identification carried out.

### RESULTS

The results obtained in case of Malta and Sangtra oranges and even of different varieties of Malta orange are in general the same excepting their storage life. Consequently the data mainly of one variety, viz. Common (and of other varieties wherever necessary) are presented to economize space. The data relating to storage life of different varieties of Malta and Sangtra orange are given in each case. The temperature range of 29°-32° F. being absolutely unsuitable for the storage of oranges due to the development of chill spots, the data at this temperature range were not collected.

# EFFECT OF STORAGE TEMPERATURE, SIZE OF FRUIT, WRAPPING AND PERIOD OF STORAGE

## GENERAL CONDITION AND WASTAGE OF THE FRUIT

At 29°-32°F. the fruit developed chill spots (Plate XXX, fig. 2) after a couple of weeks in storage. The spotting was invariably accompanied by deterioration in taste to a varying degree which ranged from flat-watery to bitter and abnoxious. Even fruit, without chill spots at this temperature deteriorated in taste. This deterioration in taste was more marked when the fruit was placed at room temperature (90°-100°F.) for a few hours. Chill spot trouble was the least in case of Valencia Late and the most in Blood Red.

36°-39°F. proved to be the best range of temperature for the storage of Malta and Sangtra oranges. Malta Common at this temperature kept in excellent condition for four months (Table I), Blood Red for three months, Valencia Late for four and a half months, Seville for three months and Musambi for 2½ months (Fig. 2). Sangtra from Lyallpur and Pathankote kept in excellent condition for seven weeks in 1938 but the storage life was reduced to five and four weeks respectively in 1939 as the fruit was subjected to greater amount of handling and was not carefully picked and packed by the grower.

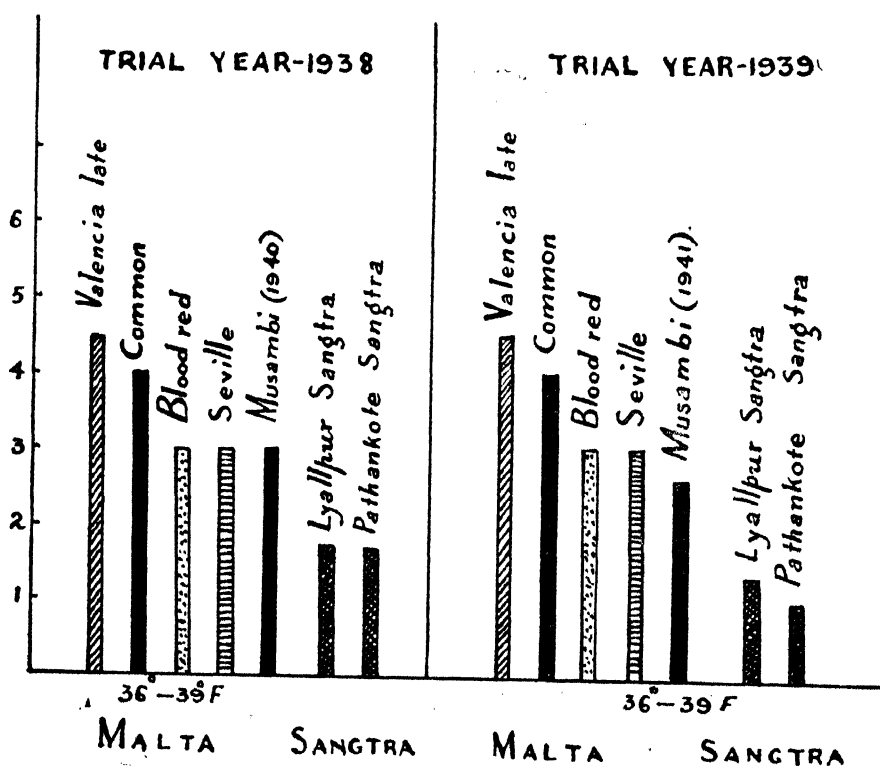


FIG. 2. Showing the maximum cold storage life of different varieties of Malta orange and Sangtra under optimum conditions

The storage life of the Malta orange of different varieties was reduced at 40°-43°F. both due to fungal attack and shrivelling.

The size of the fruit had considerable effect on the storage life of the fruit, large sized fruit kept in good condition for a longer period as compared to small ones (Fig. 3).

Small fruits presented shrivelled appearance earlier than the large fruits and storage life was thus considerably shortened in case of small fruits (Table I). Similarly, wrapping the fruit with butter paper proved to be beneficial in preserving the colour, brilliancy and freshness of the skin of all the varieties tried.

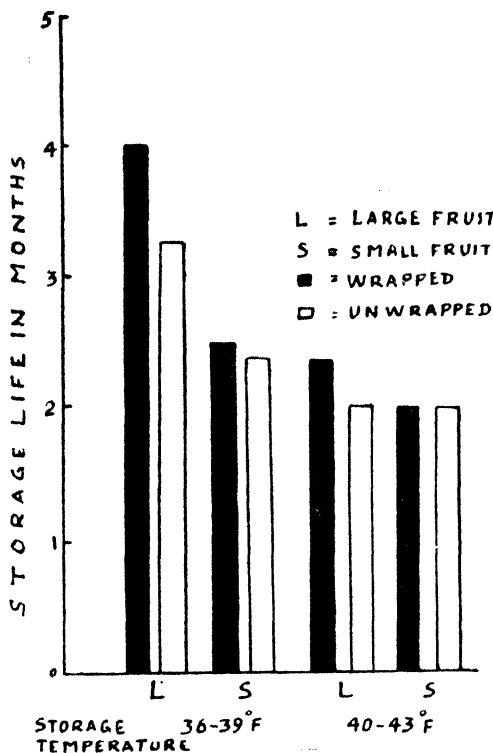


FIG. 3. Showing the storage life of Malta common fruit under different treatments.

#### LOSS IN WEIGHT OF FRUITS (Tables II, III, IV & V)

The per cent total loss in weight of fruit (calculated on original fresh weight of fruit) was determined from time to time. It was observed that the loss in weight increased with the advance in storage period. The loss in weight was the greatest at 40°-43°F. and the least at 29°-32°F. and unwrapped fruit lost more weight than wrapped one. Similarly, small fruit lost comparatively more weight than large one. At the end of four months storage the total loss in weight of fruit under optimum conditions (large wrapped fruit, stored at 36°-39°F.) varied from 18 per cent (in Valencia Late) to 25 per cent (in Common Malta). Blood Red lost 25 per cent of its weight after three months (Table III).

## PHYSICO-CHEMICAL CHANGES

Physico-chemical analyses of fruit were carried out for per cent weight of peel, per cent weight of juice, acidity and total soluble solids.

TABLE I

*Showing the effect of temperature, size of fruit, wrapping, and period of storage on the per cent rate of wastage of fruit, of Malta Common orange*

(Calculated on the actual number of fruits in storage)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
0 . . . . .	0	0	0	0	0	0	0	0
30 . . . . .	0	2.78	1.39	4.16	0	1.39	0	1.39
45 . . . . .	0	0	1.52	0	1.49	0	0	0
60 . . . . .	0	3.07	1.54	4.69	1.52	3.03	1.49	1.52
75 . . . . .	0	10.30	0	18.70	6.86	35.60	9.84	30.00
90 . . . . .	1.54	9.61	5.08	17.40	17.87	13.16	23.64	40.49
105 . . . . .	3.57	54.78	33.30	72.72	58.55	...	67.50	...
120 . . . . .	7.40	...	17.65	...	75.0	...	60.60	...

TABLE II

*Showing the effect of temperature, size of fruit, wrapping and time of storage on the per cent total loss in weight of Malta Common orange fruit*  
(Calculated on original fresh weight)

No. of days in storage	Large						Small					
	Wrapped			Unwrapped			Wrapped			Unwrapped		
	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.
0	0	0	0	0	0	0	0	0	0	0	0	0
30	2.6	4.15	5.15	3.22	5.33	6.10	3.04	4.84	5.82	5.16	6.45	7.00
45	...	8.63	9.04	...	9.88	10.13	...	9.97	8.71	...	10.72	12.08
60	...	12.82	14.27	...	13.35	14.20	...	14.50	15.20	...	15.23	16.71
75	...	19.80	22.40	...	20.18	23.40	...	21.48	22.35	...	22.10	24.20
88	...	21.72	27.50	...	23.32	28.10	...	23.50	27.38	...	25.0	29.10
104	...	24.05	32.00	...	26.57	33.50	...	26.02	31.50	...	28.66	34.82
120	...	27.10	35.87	...	29.83	38.37	...	31.48	35.94	...	33.30	41.70

TABLE III

*Showing the mean per cent total loss in weight of large sized fruit of different varieties of Malta orange under optimum conditions at the end of storage life*

Varieties	Storage life	Year	
		1938	1939
		36°—39°F. (per cent)	36°—39°F. (per cent)
Common . . . . .	4 months . . . . .	25.5	27.1
Valencia Late . . . . .	4½ months . . . . .	19.1	17.7
Blood Red . . . . .	3 months . . . . .	25.5	21.3
Seville . . . . .	3 months . . . . .	22.6	21.9
Musambi* . . . . .	2½ months . . . . .	23.5	19.84

\* Musambi was tried during 1940 and 1941

TABLE IV

*Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent loss in weight of fruit of Blood Red orange*

(Based on six numbered fruits)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
15 . . . . .	4.8	4.16	4.5	6.97	5.97	6.66	5.85	8.70
30 . . . . .	11.77	12.50	9.10	11.65	11.65	13.58	16.67	14.29
45 . . . . .	13.70	16.66	15.90	16.98	16.28	21.63	20.22	22.57
60 . . . . .	17.64	20.92	18.18	21.93	20.94	24.02	24.22	26.86
75 . . . . .	21.50	25.00	25.00	27.91	23.26	29.43	27.78	28.58
90 . . . . .	25.48	29.17	31.80	39.53	27.91	35.43	36.10	37.14
105 . . . . .	28.50	33.17	35.80	44.34	32.81	39.56	38.30	42.45

TABLE V

*Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent loss in weight of fruit of Valencia Late orange*

(Based on six numbered fruits)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
15 . . .	0.5	2.20	0.9	1.20	2.50	2.50	1.20	2.70
30 . . .	2.10	2.40	2.30	2.60	3.12	4.00	2.80	4.97
45 . . .	4.25	6.00	4.40	6.20	5.12	10.00	5.10	8.10
60 . . .	6.08	8.00	6.90	8.90	7.09	15.50	8.90	17.50
75 . . .	8.51	11.11	11.11	13.40	12.80	18.00	10.30	21.60
90 . . .	11.70	15.50	15.50	17.38	13.02	20.80	14.80	22.80
105 . . .	14.90	17.78	20.00	21.74	15.38	21.90	18.00	24.30
120 . . .	16.50	19.70	21.00	23.20	18.00	23.00	21.30	26.20

(i) *Per cent weight of peel* (Tables VI-VIII, Fig. 4). The per cent weight of peel was calculated on original fresh weight of fruit [Martin, 1937]. It decreased with the advance in the period and the decrease was more at 40°-43°F. than at 36°-39°F. The per cent weight of peel was higher in large fruits than in small ones to start with, and remained so throughout the period of storage. The weight of peel decreased more in unwrapped fruits than in wrapped ones.

(ii) *Per cent weight of available juice* (Tables IX-XI, Fig. 4). Figures of available juice in the fruit were calculated on the original fresh weight of the fruit [Martin, 1937]. The per cent weight of juice decreased with the advance in the storage period. The fruits stored at 36°-39°F. had higher juice content than those at 40°-43°F. after a storage of four months. Small fruits had higher juice content than large ones, at the beginning of storage, but lost more weight of juice than large ones at the optimum storage temperature, i.e. 36°-39°F. Wrapped fruits had higher juice than unwrapped fruits.

(iii) *Acid and total soluble solid contents of the juice* (Tables XII and XIII). The acid and total soluble solids decreased during the period of storage when calculated on the original fresh weight. Other treatments,

viz. size, wrapping and temperature did not exhibit any marked differences in the total soluble solid content though acid contents at 40°-43°F. were lower than that at 36°-39°F. after four months storage.

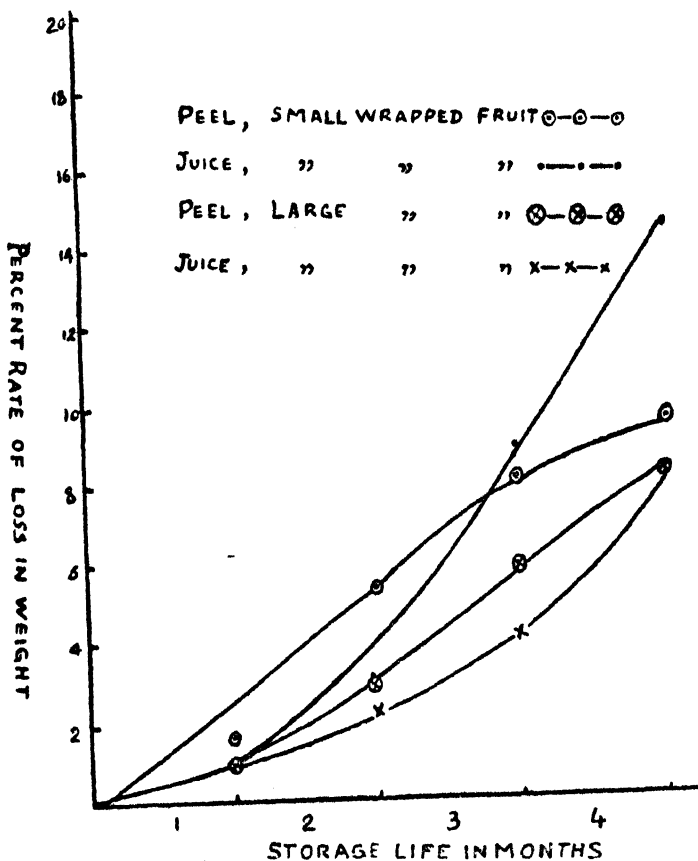


FIG. 4. Showing comparative loss in weight of peel and juice of Malta orange during storage under optimum conditions

#### FUNGAL PATHOGENS

##### *Malta oranges*

Chill spotting and deterioration in taste were mainly responsible for spoilage at low temperature, viz. 29°-32°F. But at higher temperatures (36°-39°F. and 40°-43°F.) the wastage was mostly due to the fungal attack, viz. *Penicillium digitatum* and *P. italicum*. The first symptom was the softening of the tissue followed by a visible spread of the fungus. *Alternaria* sp. was also isolated from a few fruits. The fungus in this case was observed on the internal segments of the pulp near the stem end. In 1939 storage trials, *Colletotrichum gleosporioides penzig* was also observed to cause the stem end rot.

TABLE VI  
*Showing the effect of temperature, size of fruit, wrapping and time of storage on the per cent weight of peel of Malt Common orange fruit*  
 (Calculated on original fresh weight)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.
0	34.48	34.48	34.48	34.48	28.15	28.15	28.15	28.15
30	33.57	33.48	32.32	33.01	27.50	24.82	28.40	24.38
60	...	31.50	26.20	...	...	22.57	...	20.04
90	...	28.56	21.01	...	...	19.83	...	17.85
120	...	25.95	17.55	...	...	18.33	...	15.82

TABLE VII  
*Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent weight of peel of Blood Red orange fruit*  
 (Calculated on original fresh weight)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
0	34.03	34.03	34.03	34.03	31.5	31.5	31.5	31.5
45	28.5	27.85	27.5	25.1	26.02	23.80	20.30	22.97
75	24.20	24.02	22.1	20.20	21.50	18.90	19.30	18.90
105	21.42	19.08	17.36	14.35	17.60	14.97	15.88	13.5

TABLE VIII  
*Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent weight of peel of Valencia Late orange*  
(Calculated on original fresh weight)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	29°-32°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.
0	34.52	34.52	34.52	34.52	29.78	29.73	29.78	29.78
30	...	34.68	...	32.58	...	27.78	...	27.30
60	...	29.64	...	28.77	...	27.40	...	26.78
90	...	28.59	...	25.34	...	25.10	...	24.88
120	...	26.20	...	24.12	...	21.45	...	19.90

TABLE IX

*Showing the effect of temperature, size of fruit, wrapping and time of storage on the per cent weight of available juice of Malta Common orange fruit*

((Calculated on original fresh weight))

No. of days in storage	Large				Small				
	Wrapped		Unwrapped		Wrapped		Unwrapped		
	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.
0	44-13	44-13	44-13	44-13	44-13	44-13	52-07	52-07	52-07
30	43-55	43-27	43-42	43-25	42-80	42-60	50-85	51-20	49-34
60	...	42-00	40-70	...	39-00	38-90	...	48-58	46-10
90	...	39-95	37-06	...	37-70	35-40	...	43-48	41-05
120	...	36-04	31-04	...	33-16	28-73	...	37-20	33-85

TABLE X

*Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent weight of available juice of Blood Red orange*

(Calculated on original fresh weight)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
0 . . . .	48.91	48.91	48.91	48.91	51.4	51.4	51.4	51.4
45 . . . .	43.15	41.55	41.9	41.2	43.78	44.2	44.9	45.1
75 . . . .	40.56	37.78	38.7	36.64	41.2	40.45	40.5	40.6
105 . . . .	36.13	34.57	33.43	29.34	38.4	32.08	34.25	31.4

TABLE XI

*Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent weight of available juice of Valencia Late orange*

(Calculated on original fresh weight)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
0 . . . .	48.7	48.7	48.7	48.7	52.77	52.77	52.77	52.77
30 . . . .	49.0	46.4	46.5	46.36	53.1	52.0	52.5	52.0
60 . . . .	48.4	47.20	48.2	44.6	50.14	46.4	48.7	45.6
90 . . . .	45.09	44.8	44.37	44.6	47.02	45.19	46.5	44.16
120 . . . .	44.73	42.5	42.2	41.7	47.0	45.4	45.2	43.53

TABLE XII

*Showing the effect of temperature, size of fruit, wrapping and time of storage on the acid contents of Malta Common orange fruit*

(Calculated on original fresh weight)

(Acidity given in grammes of citric acid per 100 c.c. juice)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	29°-32°F.	40°-43°F.	20°-32°F.	36°-39°F.	40°-43°F.	36°-39°F.	29°-32°F.	40°-43°F.
0	0.67	0.67	0.67	0.67	0.67	0.64	0.68	0.68
30	0.60	0.55	0.65	0.64	0.49	0.67	0.60	0.58
60	...	0.54	...	0.53	0.55	...	0.55	0.53
90	...	0.51	...	0.54	0.45	...	...	0.48
120	...	0.44	...	0.56	0.44	...	...	0.41

TABLE XIII

*Showing the effect of temperature, size of fruit, wrapping and time of storage on the total soluble solids of juice of Malta Common orange fruit*  
(Calculated on original fresh weight)

No. of days in storage	Large				Small				
	Wrapped		Unwrapped		Wrapped		Unwrapped		
	29°-32°F. (per cent)	36°-39°F. (per cent)	40°-43°F. (per cent)	29°-32°F. (per cent)	36°-39°F. (per cent)	40°-43°F. (per cent)	29°-32°F. (per cent)	36°-39°F. (per cent)	40°-43°F. (per cent)
0	9.30	9.30	9.30	9.30	9.30	9.30	9.80	9.80	9.80
30	8.57	9.30	9.58	8.33	8.08	9.70	9.31	9.84	8.60
60	...	8.11	8.32	...	8.14	8.72	...	...	9.15
90	...	8.45	7.97	...	8.43	8.29	...	...	7.80
120	...	7.29	8.08	...	8.11	8.75	...	...	7.85

## SANGTRA ORANGES

In Sangtra, in addition to blue and green moulds, another fungus *Aspergillus niger* was also found on decaying fruits.

## KEEPING QUALITY OF THE FRUIT AFTER REMOVAL FROM COLD STORE

Malta oranges were occasionally removed from cold store and placed at room temperature (86°-110°F.) to see the keeping quality of the fruit after removal from the cold store. It was observed that the keeping quality of fruit at room temperature was dependent upon the period of storage and the temperature of the room at which the fruit was stored. The longer the period, the fruit was kept in cold store, the shorter was its keeping quality. Again the higher the room temperature at which the fruit was placed after removal from the cold store, the shorter it kept in marketable condition. The observations are given in Table XIV.

TABLE XIV

*Effect of temperature and period of storage on the keeping quality of Malta orange fruit after removal from cold store*

Room temperature at which the fruit was placed	Period of storage after which the fruit was removed from cold store	Storage temperature of fruit	Keeping quality of fruit after removal from cold store
86°F.	1 month	40°-43°F. 36°-39°F. 29°-32°F.	12 days 12 days 2 days in case of unfrozen fruit. Fruit bitter
100°-108°F.	2 months . . .	40°-43°F. 36°-39°F. 29°-32°F.	10 days 7 days Fruit bitter in taste
102°-110°F.	3 months . . .	40°-43°F. 36°-39°F.	5 days 5 days
99°-110°F.	4 months . . .	36°-39°F.	3—4 days
100°-110°F.	4½ months . . .	36°-39°F.	2—3 days

## DISCUSSION OF THE RESULTS

In the present investigations the best range of temperature for the storage of Malta and Sangtra oranges was found to be 36°-39°F. (air temperature of the cold chamber). As already mentioned under 'review of literature', the findings of various workers as to the optimum temperatures for storage of oranges vary from 32°-50°F., depending upon various considerations. While variations above and below the recommended temperatures are bound to occur due to different kinds and varieties of citrus fruits, difference in soil, climate, cultural operations, age of trees, stage at which fruit is picked, care

in handling and storage conditions, etc. yet, 36°-39°F. temperature may be taken as quite safe for the storage of Malta and Sangtra oranges under the Punjab conditions.

At the lower temperature range (29°-32°F.) the fruit showed signs of skin collapse—a malady known as 'chill spot' injury of the fruit. The fruit thus affected lost its market value. In addition to this, the fruit at this temperature tasted bitter, probably due to the liberation of the bitter principle in oranges the 'Limonin' [Highby, 1941].

At higher temperatures (40°-43°F.) the storage life of the fruit was considerably shortened due to the appearance of fungal diseases and shrivelling of the fruit.

The size of the fruit is an important factor in determining the storage life of the fruit. The present investigations reveal that large sized fruit kept much longer and in better condition and lost less weight than small sized fruit. This is in conformity with the results obtained by other workers referred to under 'review of literature'.

Wrapping the fruit with butter-paper proved beneficial in reducing the loss in weight of fruit and also preserved the brilliancy and freshness of the fruit. No 'suffocation' of the fruit resulting in deterioration of taste as mentioned by Williams [1938] was observed in wrapped fruit as the outer covering of the butter-paper was not so air-tight as to completely obstruct the exchange of gases. Another advantage of wrapping the fruit was that the fruit getting diseased remained isolated from healthy ones. Wrapping of the fruit has also been recommended by other research workers, referred to under 'review of literature'.

The storage life of different varieties of Malta orange was found to vary considerably. Malta Common kept well for four months, Valencia Late 4½ months, Blood Red three months, Seville three months and Musambi 2¾ months under optimum conditions of storage (36°-39°F.). The study of literature on the subject reveals that storage life of even the same variety, varies under different conditions and thus no absolute limit can be laid down as to the actual storage life of the fruit. The present investigations, therefore, indicate the limits around which the storage life of a particular variety would oscillate as the extent of storage life is influenced by so many factors, already mentioned.

The storage life of the Punjab Sangtra orange was considerably shorter (4-7 weeks) than Malta orange which is probably due to the 'puffy' nature of the fruit, which makes it liable to damage very easily in transit or in handling. The storage life of King orange has been reported to be 50 days at 45°F. and of Nagpur orange 90 days at 40°F. but these varieties are not cultivated in the Punjab and are far more tight skinned than the Punjab Sangtra orange.

Physico-chemical analyses of the fruit showed that the loss in weight was both from peel and juice during the storage period. In the beginning of the storage life, peel lost weight to a greater extent than the juice, while at the end of the storage life reverse was the case. This probably is due to the fact that peel being more turgid in the beginning, readily lost its moisture before the juice could be affected. Small fruits lost more weight than large ones as has been observed by other workers, also, referred to under 'review of literature'.

This is due to the fact that in small fruits, the surface exposed per unit of volume is more than in large fruits.

The acid and total soluble solid contents of the juice were not affected in proportion to the decline in taste and consequently only acid and total soluble solid contents cannot be true index of quality. The acid and total soluble solids showed a decrease during the period of storage when calculated on original fresh weight and this is also observed by other workers cited under 'review of literature'.

Fruit removed from cold storage and placed at room temperature showed that the keeping quality of the fruit at room temperature decreased with the advance in the period of storage. This is probably due, partly to the rise in temperature of the room during summer and partly to low resistance of the fruit to withstand high temperatures after prolonged storage at low temperature.

#### SUMMARY

The investigations reported in the paper were carried out during 1938 and 1939 at Lyallpur under the Research Scheme on the Cold Storage of Fruits in the Punjab, financed jointly by the Imperial Council of Agricultural Research and the Punjab Government. Results of investigations on Malta (*Citrus sinensis*) and Sangtra (*C. nobilis*) obtained during the above period may be summed up as under :—

1. Five varieties of Malta, viz. Valencia Late, Common, Blood Red, Seville and Musambi and Sangtra from two localities namely Lyallpur and Pathankot were stored at three storage temperatures, viz. 29°-32°F. 36°-39°F. and 40°-43°F. Large and small fruits of each variety were used. Half of the fruit was wrapped with butter-paper and the other half stored as such.

2. Physico-chemical analyses were carried out at regular intervals.

3. The best temperature range for the storage of citrus fruits (Malta and Sangtra) was found to be 36°-39°F.

4. The storage life of Malta (*C. sinensis*) varied with varieties, (a) Valencia Late kept in good condition for 4½ months, (b) Common for four months, (c) Blood Red for three months, (d) Seville for three months and (e) Musambi for 2½ months.

5. Loose skinned Sangtra from Lyallpur and Pathankot kept in good condition for five and four weeks respectively.

6. Large fruit kept longer and in better condition than small fruit.

7. Wrapped fruit presented better appearance in regard to its colour and freshness and had higher juice content and lower wastage than unwrapped fruit.

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**CAJANUS OBCORDIFOLIA SINGH**  
A NEW SPECIES OF *CAJANUS*

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(With six text-figures)

**CAJANUS** is a monotypic genus, represented in India by the only species *Cajanus cajan* Millsp. (*Cajanus indicus* Spreng.—Hindi—*arhar*). The two sub-species are *C. indicus flavus* and *C. indicus bicolor*, the differentiation being based on the colour of the flower and the habit of the plant. This plant is extensively cultivated in the Gorakhpur district of the United Provinces. In December 1939 the senior author noted in one of the *arhar* fields a plant which was distinctly different from the others, and yet, it appeared to resemble *Cajanus*. At that time all branches except one were flowerless. The plant was allowed to seed, which was later on collected and brought to Cawnpore and sown in the Botanical Garden of the Agricultural College. For comparison a plot of normal type of *arhar* was also sown.

All the plants of this new type, which were about 50 in number, repeated without exception the characters of the mother plant. The chief characters of the new type of *arhar* and that of the normal *C. cajan* Millsp. are summarized in Table I given below :—

TABLE I

Character		
Leaflets	Trifoliate	Trifoliate
Shape	Lanceolate	Obovate
Apex	Acute to slightly acuminate	Retuse and mucronate
Glandular hair	Numerous and prominent	Comparatively very few
Flower petals	Yellow	Yellow ; lighter in colour
Keel	United at the top	Quite free in open flower, filiform and usually appendaged
Alae	Lobes one sided and asymmetrical with a pronounced peg-like out-growth at the base ; veins more prominent.	Lobes present on both the sides and hence symmetrical in shape, with a less pronounced peg-like out-growth at the base ; veins comparatively inconspicuous

It is evident from the comparative description that the new type differs from the normal type in having small and obcordate leaflets with retuse and mucronate apices, while the normal type has oblong lanceolate leaflets (Figs. 1 and 2). On the basis of 100 observations made regarding the central leaflet, the following interesting data have been obtained and are shown in Table II and Fig. 3.

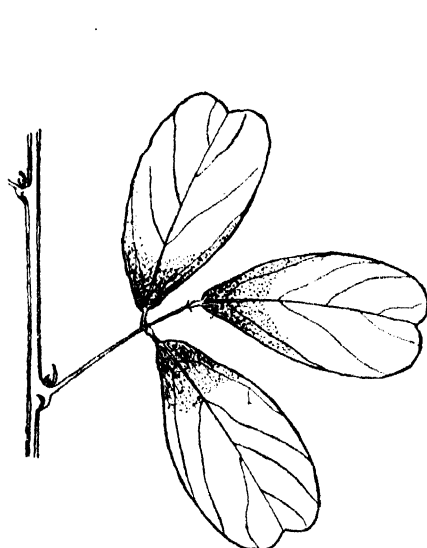


FIG. 1. Showing *Cajanus* (New type)

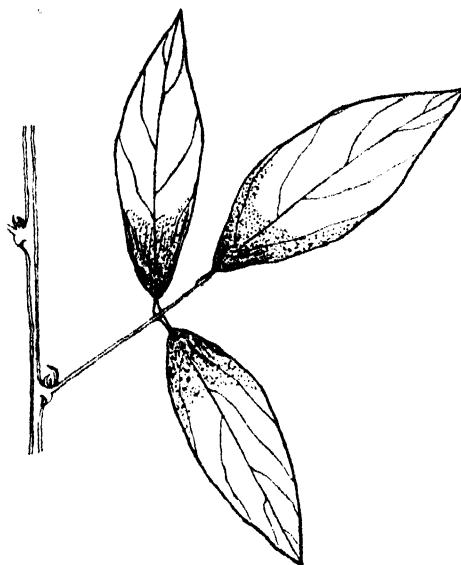


FIG. 2. Showing *Cajanus cajan* (L.)  
Millsp. (*Cajanus indicus* spreng.)

TABLE II

Name	Variation in the length of central leaflets in mm.	Mean length of central leaflet in mm.	Variation in the width of central leaflets in mm.	Length Width ratio
<i>C. cajan</i>	89-110	97.2	35-43	2.3-2.8
<i>C. obcordifolia</i>	63-76	68.6	31-40	1.5-2.1

The structure of the flowers is also very different ; the keel in the new type of *arhar* is free and represented by two filiform lobes usually appendaged while in the normal type the lobes are broad and united at the top (Figs. 4, 5 & 6). The alæ or wings in the normal type are one-sided and asymmetrical having a very pronounced peg-like outgrowth at the base, whereas, these in the new type are symmetrical having lobes on both the sides with a less pronounced peg-like out-growth.

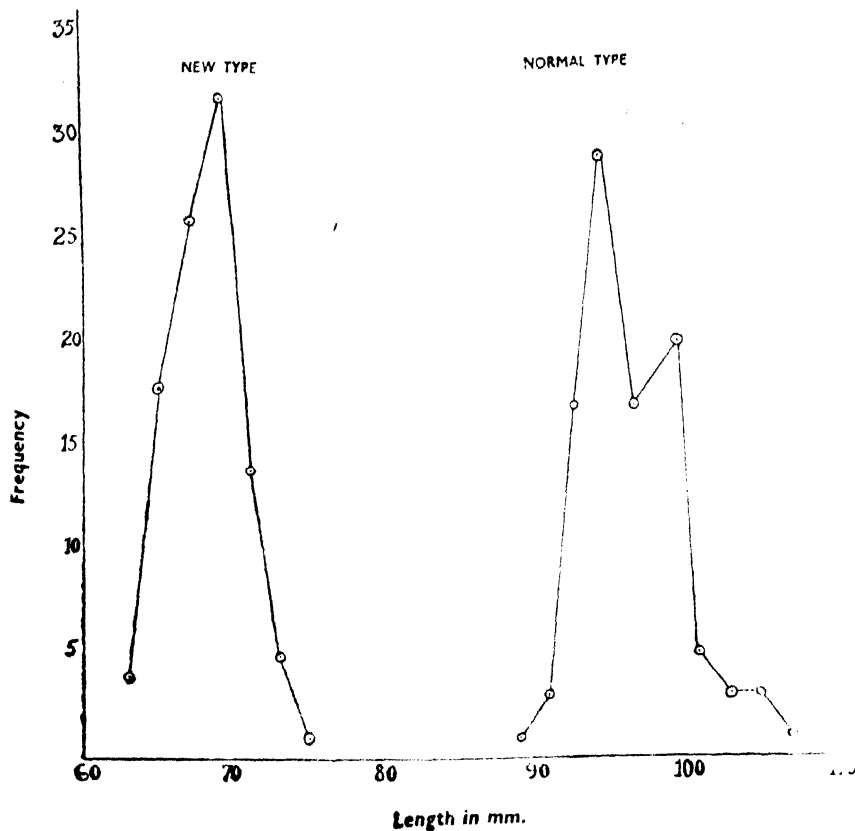


FIG. 3. Showing frequency distribution of central leaflet length in normal and new type



Keel petals free      Keel petals united      Keel petals new type  
 FIGS. 4, 5 and 6. Showing the structure of flowers of *Cajanus* (New type) and *Cajanus cajan* (L.) Millsp. (*Cajanus indicus* Spreng.)

According to analytical key given by Bailey [1924] the plant should belong to the genus *Cajanus*. The description of *Cajanus indicus* Spreng., given by Hooker [1875] states that the leaflets are oblong—lanceolate; Bailey states that they are lanceolate to narrow—elliptical. Hooker's classification is silent about the keel, while Bailey describes it as 'obtuse and incurved'. The keel is a very distinctive character of Leguminosæ—Papilionaceæ. Had any deviation from the normal keel been noted in *Cajanus cajan* it would have been emphasized. But so far no reference in the literature on the subject is made to this fact.

During the course of above investigation, a study of the chromosome number of this new type was also made. Aceto-carmene smears of pollen mother cells were prepared for determining the gametic chromosome number. Buds of about 3 mm. in length were found to be the best for the study of the meiotic chromosomes in the pollen mother cells at the first metaphase. The haploid number of chromosomes was found to be eleven.

The study of the somatic chromosomes from the root-tips of the germinating seeds was also made. The root tips were fixed in Flemming's chromosmium-acetic combination (weaker solution) and after the usual processes of embedding and cutting, the sections were stained by Newton's Gentian Violet Iodine method and mounted in Canada balsam. The number of somatic chromosomes was found to be  $2n=22$ .

This new type of *Cajanus* may be the result of gene-mutation. Further investigations on other features of genetical and cytological interest are in progress. In the meanwhile owing to certain marked differences in vegetative and floral characters in this new type of *Cajanus*, it may be considered as a new species and is tentatively named as *Cajanus obcordifolia* Singh after its more distinguishing character, viz. the obcordate shape of the leaflet.

Botanical description of *Cajanus obcordifolia* Singh. in English

- Plant : . . . Erect and shrubby
- Root : . . . Tap ; fibrous and branched
- Stem : . . . Erect ; woody ; cylindrical and ribbed with many slender, sulcate and grey silky branches
- Stipule : . . . Minute ; lanceolate ; fugacious
- Leaf : . . . Alternate ; compound ; imparipinnate ; with channeled long petiole ; trifoliate
- Leaflet : . . . Stalked, minutely stipellate, reticulate, obcordate with retuse and mucronate apex ; margin entire ; glabrous ; deep green above and whitish and pubescent below
- Inflorescence : . . . Indefinite ; corymbose raceme ; often forming a terminal panicle
- Flower : . . . Irregular ; hermaphrodite ; pedicellate ; pedicels profusely hairy and two to three times the calyx. The floral bud has a crumpled tip and not unoften there is an opening at the extreme tip
- Calyx : . . . Gamosepalous ; campanulate ; persistent ; glandular ; pubescent ; teeth short ; inferior

- Corolla : . . . Irregular ; polypetalous ; papilionaceous ; more than twice as long as the calyx ; perigynous ; standard yellow ; alæ or wings with lobes on both the sides and more or less symmetrical in shape ; peg-like outgrowth (auricle) at the base less pronounced ; clawed ; veins inconspicuous. Keel petals light in colour ; Quite free in open flower ; filiform and usually appendaged
- Andrœcium : . . . Ten in two bundles (9) + 1 (diadelphous) ; not enclosed in keel in open flower ; anthers uniform. These dehisce in the bud even before the flower has reached its maximum size. This early dehiscence in this type may be due to the small opening at the tip resulting in greater loss of moisture from the anther walls
- Gynœcium : . . . Monocarpellary ; subsessile ; few ovuled ; superior ; with a long filiform and upcurved style ; stigma capitate ; ovary wall pubescent ; pod with conspicuous black splashes (markings) ; beaked and constricted between the seeds ; hairy ; seed exalbuminous ; compressed ; smooth and of light colour

Botanical description of *Cajanus obcordifolia* Singh. in Latin

*Cajanus obcordifolia* Singh. sp. nov.

Frutex erectus. Radix principalis fibrosa et ramosa. Truncus erectus ligneus, cylindricus et multis tenuibus, sulcatis et cinereo-sericeis ramis costatus. Stipuli minuti, lanceolati, decidui. Folia alterna, composita, imparipinnata longo sulcato petiolo prædita, trifoliata. Foliola petiolata, minute stipellata obcordate, apice retuso et mucronato ; margine integræ ; superiore facie glabra et valde viridi, inferiore vero albescente et pubescente. Inflorescentia indefinita, corymbose racemosa ; sæpe terminaliter paniculata. Flores irregulares, hærmaphroditi, pediculati ; pediculi profuse pilosi, et bis vel ter calyce longiores. Floris gemma est corrugata apice et non raro in extremo apice foramine prædita. Calyx gamosepalus, campanulatus, persistens, glandulis præditus pubescens, inferior ; calycis dentes breves. Corolla irregularis, polypetala, papilionacea, plus bis longior calyce, perigyna ; vexillum flavum ; alæ lobatæ in utroque latere et plus minus symmetricæ forma ; auricula clavo similis in basi minus conspicua ; petala ad basim tenuescencia ; venæ inconspicuæ. Carinæ petala colore claro, omnino libera in aperto flore, filiformia et sæpe sæpius appendiculata. Andrœcium ; stamina diadelphe (9 + 1) ; non inclusa in carina in aperto flore ; antheræ uniformes, dehiscentes in gemma etiam antequam flos maximam magnitudinem attigerit. Præmaturæ huius dehiscentiæ causa forte sit parvum illud foramen in apice gemmæ, quod efficit ut antheræ parietes majorem humiditatis quantitatem amittant. Gynœcium monocarpum, subsessile, superius, paucis ovulis et stylo longo filiformi et sursum curvato præditum ; legumen conspicuis nigris maculis ornatum, rostratum et constrictum inter semina ; pilosum. Semen exalbuminatum, compressum, planum vel læve et colore clarum.

#### ACKNOWLEDGEMENTS

Our thanks are due to Dr T. S. Sabnis, I.A.S., Economic Botanist to Government, United Provinces, and Principal, Agricultural College, Cawnpore,

and Mr P. R. Mehta, Assistant Professor of Botany, for their helpful suggestions, and to Mr. T. R. Mehta for his unfailing interest and kind criticism during the course of the above investigation. We feel greatly indebted to Rev. Fr. Santapan of the St. Xavier's College, Bombay, for the Latin description of the new species.

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# RESEARCH NOTE

## CHROMATIN BRIDGES IN COTTON\*

BY

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(Received for publication on 7 January 1942)

(With four text-figures)

CHROMATIN bridges were noticed at anaphase I, metaphase II and anaphase II of meiosis (Figs. 1-4) in  $F_1$  triploid hybrids between Asiatic and American cottons shown in the table on the next page.

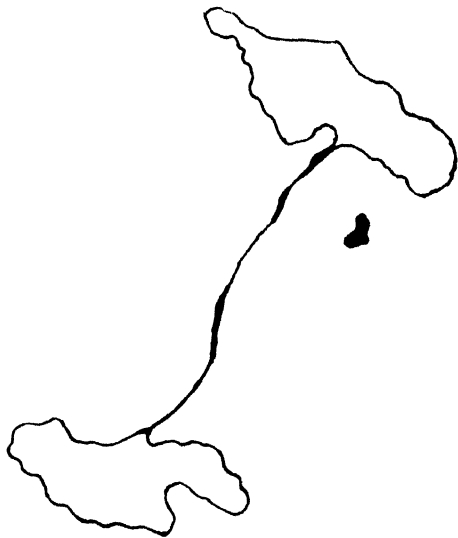


FIG. 1. A bridge and a fragment at anaphase I ( $\times 2375$ )



FIG. 2. A bridge at metaphase II ( $\times 2750$ )



FIG. 3. Three bridges at metaphase II ( $\times 2375$ ) (all chromosomes not shown)



FIG. 4. A bridge and a fragment at anaphase II ( $\times 2750$ )

\*This research is being financed from the funds of the Indian Central Cotton Committee

Hybrid No.	Parents	
	American	Asiatic
S 29-1 . .	<i>G. barbadense</i> L (Sea Island)	<i>G. herbaceum</i> var. <i>frutescense</i> (Surat 1027 A L F)—Delile
S 31-3 . .	"	"
S 38-1 . .	"	"
S 37-1 . .	<i>G. hirsutum</i> L (Coimbatore 2)	"

These bridges are clearly seen in acetocarmine smears of the flower buds at metaphase II (Figs. 2 and 3). The number of such bridges at this stage varied from one to four per nucleus, indicating that structural changes have taken place in more than one bivalent. The bridges are very similar to those figured by Miduno [1940] in the orchids and Srinath [1940] in the genus *Calceolaria*.

The formation of bridges at anaphase have been reported by Beasley [1940] in the F1 hybrid between *G. thurberi* Tod.  $\times$  *G. arboreum* var. *neglectum* H. & G., and by Ramiah and Gadkari [1941] in a sterile mutant of a strain of Asiatic cotton (*G. arboreum* var. *neglectum* forma *burmanica* H. & G.). Beasley [1940] has also pointed out that such bridge formations give evidence of structural differences between the chromosomes of the species involved in his cross. That definite structural changes, like inversions and translocations could have taken place in cotton has been pointed out by Jacob [1941], where he shows one of the two chromosomes with a lateral satellite in the root-tip of *G. herbaceum* and *G. arboreum* and a ring chromosome in the root-tip of *G. herbaceum* var. *africanum* H. & G., at the metaphase stage. Further evidences that definite structural differences do exist between the chromosomes of certain species of cotton are pointed out in this note.

The formation of bridges may be due to several causes as inversions, translocations and duplications [Richardson, 1936]. A critical analysis of both metaphase and anaphase at division I is necessary to enable us to say what conditions have given rise to observed results and which of the structural changes that are possible have taken place. In the hybrids that are examined in the present study no abnormal configurations at metaphase I, as unequal bivalents etc. could be clearly made out. The bridges that are seen at metaphase II are long and thin and their persistence at this stage shows that the bridges formed at anaphase I are not broken. The fragments that arise when such bridges are formed are difficult to make out in all the cases. The formation of a bridge at anaphase II, in one of the sister cells (Fig. 4), undoubtedly indicates that a loop chromatid must have been formed at anaphase I, as a result of an inversion pairing and two cross-overs having taken place, one in the inversion region and one in the region proximal to it, in which only one chromatid is involved in both the cross-overs. A monocentric loop and a fragment would be formed at anaphase I. The loop chromatid forms a bridge at anaphase II, the centromere having divided.

The above points indicate that in the triploids under study, we not only deal with mere numerical changes but structural changes as well. Both of these factors may contribute to the sterility of the hybrids. All the same the structural changes lead to the formation of new chromosomes which may prove to be of evolutionary significance.

A fuller discussion of the above points will be published in due course.

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# PLANT QUARANTINE NOTIFICATIONS

NOTICE No. 2 OF 1942

## INDIA

**T**HE following plant quarantine regulations and import restrictions have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

### 1. U. S. Department of Agriculture, B. E. & P. Q.

#### 1. *Summaries of plant quarantine import restrictions.*

(i) Republic of Cuba—Revision of regulations.

(iii) Republic of Uruguay—Standards established for Certain seeds.

(iii) Br. Colony of Malta—Area quarantined on account of Colorado Potato Beetle increased.

#### 2. *Service and Regulatory Announcements.*

List—October—December 1941.

### II. Other Announcements.

*Jamaica—Br. W. I. : Citrus fruits in ports.*

*Notification No. 69/C. No. 429-Cus. II/41, dated 20 December 1941 of the Government of India in the Department of Finance (Central Revenues)*

**I**N exercise of the powers conferred by section 19 of the Sea Custom Act, 1878 (VIII of 1878), the Central Government is pleased to prohibit with effect from 1 April 1942, the bringing into British India of bees or silk-worms save where they are accompanied by—

(a) a special permit in accordance with the form set forth in the Schedule hereto annexed authorizing such importation issued by the Central Government or by an officer authorized by the Central Government in this behalf; and

(b) a certificate of freedom from disease granted by an Entomologist of the Government of the country of origin.

### SCHEDULE

*Form of special permit authorizing importation of bees or silk-worms*

1. Name, designation and full address of the importer .....
2. Name of the species of bees or silk-worms to be imported .....
3. Stage or stages of the bees or silk-worms to be imported ..
4. Country from which importation is sought.....

5. Whether importation is intended by sea, land or air .....

6. Name, designation and address of the exporter .....

7. Quantity indented for .....

8. Purpose of importation .....

The above information is true to the best of my belief

Date

(Signature of the importer)

I authorize the importation. This permit will be valid up to

Date

(Signature and designation of the certifying authority)

[N. B. It is expected that the permit will be obtained in advance of sending the order so that the imported material may not remain indefinitely in the warehouse for want of suitable permit.]

*Notification No. F. 15-21/41-A., dated 12 May 1941 of the Government of India in the Department of Education, Health and Lands*

**I**N exercise of the powers conferred by sections 4A and 4D of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government is pleased to direct that the following further amendments shall be made in the notification of the Government of India in the Department of Education, Health and Lands, No.F.50-13 (20) /39-A, dated 20 November 1940, and the rules published therewith, namely :—

I. In the preamble to the said notification, and in rule 1 of the said rules, after the word ' Punjab ', the words ' the United Provinces ' shall be inserted.

II. In the *Note* below the Schedule annexed to the said rules, clause (b) and (c) shall be re-lettered as clauses (c) and (d) respectively and before clause (c) as so relettered, the following clause shall be inserted, namely :—

' (b) in the United Provinces, by the Entomologist to the Government of the United Provinces, or such other officer as may be authorised by the Provincial Government in this behalf '.

# FOREIGN

*Notice No. 1 of 1942 regarding plant quarantine regulations and import restrictions received in the Imperial Council of Agricultural Research*

**T**HE following plant quarantine regulations and import restrictions have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

LIST OF UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, IMPORT RESTRICTIONS, SERVICE REGULATORY AND OTHER ANNOUNCEMENTS

1. *Summaries of plant quarantine import restrictions.*—

- (i) Republic of Mexico.—Substitution of quarantines regarding coffee & banana
- (ii) Republic of Uruguay.—Restriction on the importation of seed potatoes
- (iii) Union of South Africa.—Revision of regulations concerning tomato seed
- (iv) British Colony of Bermuda.—Amendment of banana prohibition
- (v) Colony of British Guiana.—Restrictions of coffee and paddy rice & prohibition of citrus

2. *Service and Regulatory Announcements.*—

- (i) List—April—June 1941
- (ii) Index—1940

3. *Other announcements.*—

Canadian Order.—in council enabling the Inspectors to withhold certificates of inspection.

*Exports of potato to Mauritius*

**I**T is notified for general information that exports of potato to Mauritius must be accompanied by a certificate stating that the potatoes have been grown in a locality free from Potato Wart (*Synchytrium edoioeloticum*) and Colorado Beetle (*Leptinotarsa decemlineata*).

## NOTE

**I**N the article entitled 'Studies on the Formation of Jellies from some Indian Fruits' by Birendra Narain Singh and Sikhibhushan Dutt published in the *Indian Journal of Agricultural Science*, Vol. XI, Part VI, December 1941, pp. 1006-21, the authors claim to have discovered the jelly-forming qualities of wood apple in the following words:--'It has, however, been found by the present investigators to be an excellent jelly-forming material, being very rich both in acid and pectin.' It has now been brought to our notice that the making of an effective wood apple jelly was first studied by Mr S. S. Bhat, Horticulturist to Government, Baroda, at the Baroda Fruit Preservation Laboratory and the results published in *Rural India*, Vol. 3, No. 3, March 1940 (Editor).



## ORIGINAL ARTICLES

### THE NATURE AND EXTENT OF DAMAGE CAUSED BY *BEMISIA GOSSYPIPHERDA* M. AND L., THE WHITE-FLY OF COTTON IN THE PUNJAB

BY

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(Received for publication on 17 July 1939)

(With Plate XXXI and two text-figures)

#### INTRODUCTORY

THE economic importance of some of the species of Aleurodidae has now been definitely recognized and a number of them are known as serious pests of flower plants, fruit trees and cultivated crops. In India some of the white-flies which are responsible for causing considerable economic loss to growers, are *Dialeurodes citri* and *Dialeurodes elongata* on citrus; *Aleurolobus barodensis* on sugarcane; *Aleyrodes ricini* on castor and *Bemisia gossypiperda* on cotton. The damage by the white-flies in the respective cases is quite similar, since unlike most other sucking insects the mechanical injury is not appreciable and yet the after-effects of the attack are very serious.

So far, no precise information, however, is available with respect to the real nature of injury caused to the infested plants. According to Berger [1910] and Morrill and Back [1911], Aleurodidae have never been accused of killing the plant tissues. This is evident even in the case of the citrus plants which are slow growing and where the white-fly infestation is carried over from year to year on the leaves of the same plant. Notwithstanding the heavy infestation in certain cases, the leaves attain almost normal size and shape but the fruit, on the other hand, is comparatively less developed and considerably reduced in number.

As a pest in greenhouses, Lloyd [1922] regarded the white-flies as responsible for lowering fruit formation as well as for desapping the vitality of the leaves and some times causing them to drop. This contention has received support from Hasement and Jones [1934], but the exact condition and age of the plants when the leaves were shed have not been described.

Virus diseases of a number of field crops have been associated with the white-flies. Golding [1930], Kirkpatrick [1930-31] and Massey and Andrews [1932] hold white-flies responsible for leaf crinkle of cotton in the Sudan,

Hopkins [1932] and Mossop [1932] regard a species of Aleurodidae as a carrier of the leaf-curl disease in tobacco at Rhodesia. Mathur [1933] regards *B. gossypiperda* as a vector of leaf-curl in zinnia at Dehra Dun, while Pruthi and Samuel [1937 ; 1939] consider the same species responsible for the transmission of leaf-curl virus in tobacco at Pusa (north Bihar).

As a pest of cotton, it has been discussed previously by Husain [1930] and Husain and Trehan [1933] that the white-fly is not capable of producing any structural malformation of foliage or any other part of the plant. At any rate, it is quite obvious that thousands of white-fly nymphs which are generally present on an infested plant, must deprive the host of its nourishment by constantly sucking the vital juices. Besides, these nymphs secrete copious quantities of honey-dew which falls on the leaves below and provides a suitable medium for the growth of a black mould. Thus the internal metabolic activities of the plant are also disturbed due to interference with its photosynthesis.

In the absence of any visible structural injury to the plant, even in cases of severe infestation, and under the conditions stated above, it was considered necessary to investigate in what manner these insects damage their hosts and bring about the resultant effects. Since it is universally recognized that fruiting in cotton plants is affected considerably by the relative disturbance in the products which are manufactured in the foliage, it was suggestive that the nature of injury might be determined by investigating the rôle of this pest in affecting the production of certain plant constituents. Moreover, the extent of damage as a result of the white-fly attack also required estimation.

Attempts have, therefore, been made to investigate the following aspects of the problem :

1. Injury caused to foliage or entire plant
2. The effect of attack on the total dry-weight of the parts of a plant produced above soil
3. The effect and after-effects of attack on :
  - (i) Growth of plant
  - (ii) Flower and boll formation
  - (iii) Lint and seed development

The present investigations were carried out at Lyallpur from 1931 to 1936 under a scheme financed by the Indian Central Cotton Committee, Bombay. The generosity of the Committee is gratefully acknowledged.

The statistical analysis of the data was carried out by Mr Dwarka Nath Nanda, Statistical Assistant, Cotton Research Laboratory, Lyallpur. His help is gratefully acknowledged.

#### TECHNIQUE

All the experiments during these investigations were carried out in big wire-gauze cages. Healthy seeds from a single plant were sown in blocks under exactly similar cultural treatments. The plants were enclosed in cages soon after the first irrigation and ultimately each cage had four to ten plants for general observations. Dry-weight experiments were performed in relatively bigger cages and, therefore, sowing was done inside them. Each

cage had about 55 plants but those under observation varied in number in different years.

In some of these cages the plants were kept almost free from white-fly attack, in others a moderate attack approximately of the same intensity as found in nature every year was maintained, while in still others a very severe infestation was kept at different periods of growth of the plants.

To produce infestation in the cages, large number of adult white-flies were introduced and allowed to multiply. To free the plants from infestation, however, removal of adults and spraying with rosin compound was resorted to.

#### PRESENT OBSERVATIONS

##### 1. *Injury caused to foliage or entire plant*

The most obvious results of white-fly attack, as already stated, are (1) desapping of plants and (2) dropping of honey-dew on the leaves on which black mould develops which consequently interferes with their photosynthetic activities.

It, therefore, seemed possible that some injury in the form of internal physiological disturbance may be responsible for the low yield of the infested plants. Nitrogen-carbohydrate relationship may be an index of such disturbance. This was, therefore, studied.

Nitrogen was estimated by Kjeldahl's method and carbohydrates by the 'difference method' after estimating ash, fat and crude fibre. The carbohydrate estimation by the 'difference method' is not considered accurate for the determination of the rôle of various sugars or of starch in boll formation. However, it was considered that comparative values of the total carbohydrates under almost identical conditions, other than the white-fly attack, may be of some interest. Fat was estimated by petrol extract in the Soxhlet apparatus and the crude fibre from the fat-free samples.

Prior to each observation, the leaves of the healthy and infested plants selected for analysis, were carefully washed with a moist pad of cloth with a view to remove, as far as possible, the black mould and the immature stages of white-flies, if present. About 24 hours after this treatment, the leaves and other parts of the respective plants were removed separately, weighed, dried and powdered. The representative samples from individual lots were then taken for analysis.

During 1931, these observations were limited to the selected samples of leaves from infested and uninfested plants. During 1932, however, such observations were made after removing the entire leaves from such plants. During 1933 and again in 1935, these comparative observations were further extended to stems, flowers and bolls as well.

The data recorded in Tables I and III indicate that, as was to be expected, the percentage of moisture was relatively higher in the leaves of the least-infested plants than in those of the heavily infested ones. Dry matter, on the other hand, was relatively greater in the infested than in uninfested plants. These results are in perfect agreement with those of Johnson [1934] who found similar condition in the case of potato plants attacked by the leaf-hopper, *Empoasca fabae*.

TABLE I  
Results of analysis of leaves of healthy and infested cotton plants, 1931

Date	Nature of infestation	Per-centage of mois-ture	Per-centage of dry matter	Weight in 100 gm. of fresh material					C/N	Remarks
				Nitrogen	Protein	Ash	Fibre	Fat		
27 July 1931	Low	79.2	20.8	0.68	4.2	2.9	1.4	1.2	16.3	Low to n Practically free from attack with about 0.19 im-mature stages per sq. in. of leaf area
3 August 1931	High	77.4	22.6	0.56	3.5	3.4	1.6	1.4	22.7	
	Low	80.5	19.5	0.49	3.1	4.5	1.2	1.2	19.4	
	High	74.5	25.5	0.48	3.0	6.1	2.0	1.5	26.8	
11 August 1931	Low	80.0	20.0	0.52	3.3	4.9	1.8	0.9	17.5	High infestation
	High	76.0	24.0	0.50	3.1	5.6	2.3	1.2	23.6	Severe attack with 68.0 immature stages per sq. in. of leaf area
26 August 1931	Low	82.5	17.5	0.64	4.0	3.1	1.9	1.0	11.7	
	High	74.5	25.5	0.38	2.4	7.8	2.7	1.2	30.0	
28 September 1931	Low	77.6	22.4	0.72	4.5	4.8	1.5	1.4	14.1	
	High	76.6	23.4	0.37	2.3	3.3	1.4	2.1	38.6	
(1) Mean value of low infestation		79.96	...	0.61	...	4.04	...	1.140	15.80	
(2) Mean value of high infestation		75.80	...	0.46	...	5.24	...	1.480	28.34	
(3) Standard error of the difference		1.298	...	0.067	...	1.011	...	0.0927	3.754	
(4) Mean value of 't'		3.205*	...	2.268	...	1.188	...	3.656*	3.340*	

\* Significant at 5 per cent level

† Significant at 1 per cent level

Further examination of the data collected during 1931 gave the following results :

The average amount of nitrogen in 100 gm. of fresh leaves of the uninfested and infested plants was 0.61 gm. and 0.46 gm. respectively. Thus, nitrogen was relatively higher in the uninfested plants by about 33 per cent.

The amount of mineral ash was about 30 per cent higher in the leaves of the infested plants. Similarly the amount of fat and carbohydrates was higher in the infested leaves. The C/N ratio, therefore, was lower in the uninfested leaves, the differences being statistically significant.

Statistical analysis of the data in 1931 showed significant differences in the percentage of moisture, fat, carbohydrates and the C/N ratio in the leaves of the uninfested and infested plants.

During 1933 more elaborate observations were made and were continued till late in the season. Total production of various constituents was calculated on the basis of the entire fresh weight of the plants and the relative transport from the vegetative to reproductive organs determined (Table II). On examination of the entire data (Table III) it was confirmed that nitrogen was relatively higher by 11.7 per cent in the foliage of the uninfested plants till the end of August. From September onward, the order gradually reversed in the foliage indicating highly significant differences but resulted, on the other hand, in the relative increase of nitrogen in the bolls of the uninfested plants. Thus a maximum increase of 58.3 per cent of nitrogen over the bolls of the infested plants was noticed in the month of November. Moreover, the total nitrogen produced per plant, on an average, was 2.80 gm. in the uninfested plants against 2.07 gm. in the infested ones. This showed an increase of 35.2 per cent in favour of the uninfested plants. Moreover, of the total nitrogen produced during the flowering and fruiting period, about 23 per cent was estimated to have been transported from the vegetative to the reproductive organs in the healthy plants against 4.6 per cent only in the infested plants (Table II). This is an important fact and probably such metabolic activities in the plant tissue result in producing fewer flowers and bolls on the infested plants.

*Mineral ash.* Till the end of September the ash constituents were relatively higher in the foliage of the infested plants and the differences were statistically significant. From October, however, the case was reversed but from November, the amount of ash increased considerably in the bolls of the uninfested plants and went to a maximum increase of 146 per cent over that in the bolls of the infested plants. Total mineral ash produced per plant, on an average, was 17.99 gm. in the uninfested and 13.17 gm. in the infested plants. Thus, about 36 per cent more ash was produced by the healthy plants. Moreover, from the middle of October to December, 11.3 per cent of the ash was transported to the bolls in the healthy plants against only 2.4 per cent in the infested ones (Table II).

*Fat.* The percentage of fat was comparatively higher in the foliage of healthy plants (practically free from white-fly attack) throughout the season and, when compared to the severely infested ones, the differences were highly significant. During November and December, however, the fat increased considerably in the bolls of the uninfested plants reaching to a maximum of



511 per cent over that in the bolls of the infested plants. Total amount of fat produced per plant, during the season, varied from 5.44 gm. in the uninfested plants to 3.03 gm. in the infested ones. The corresponding transport of fat from the vegetative to reproductive organs, during November and December, was 41.4 per cent and 6.4 per cent respectively (Table II).

*Crude fibre.* Average amount of fibre produced in 100 gm. of fresh leaves of both the uninfested and infested plants was almost equal during the season. During November, however, it increased considerably in the bolls of the uninfested plants with a maximum of 292.7 per cent higher than that in the bolls of the infested plants.

*Carbohydrates.* The percentage of carbohydrates was higher in the foliage of the infested plants than in the uninfested ones throughout the season and the differences were highly significant. On the other hand, the carbohydrates increased considerably in the bolls of the uninfested plants and the difference reached to a maximum of 452.8 per cent during December (Table III). It is probable that more of non-metabolic carbohydrates are produced in the infested plants and, therefore, are kept stocked in the foliage and do not migrate to the bolls. On the contrary, it is likely that more of metabolic carbohydrates and relatively less of non-metabolic carbohydrates are produced in the uninfested plants. Thus they are capable of taking part in the formation of bolls when migration takes place from the leaves.

Statistical analysis of the data is presented in Table IV, which shows that the differences in the percentage of moisture, fat and carbohydrates in the leaves were highly significant. Nitrogen and ash, however, showed interesting results since the differences in nitrogen in the foliage were significant from September onward and those of ash only up to the end of September. On the other hand, the data of the entire season compared collectively did not show significant differences. This is quite obvious from the trend of the figures in Table III, because in both the cases the migration of the constituents changes the balance in the foliage.

Similar experiments as above were also started in 1935, but the plants under cages were severely damaged by a violent dust and hail storm. Consequently the white-fly infestation as well as the plants under observation did not progress well.

Total nitrogen produced per plant, during the season, was 1.20 gm. in the uninfested plants and 0.95 gm. in the infested ones. At the same time, the percentage of nitrogen transported from the vegetative to reproductive organs was about 20 per cent and 12 per cent respectively.

It is quite evident from the above observations that the most serious effect of a heavy white-fly infestation is the reduction in the amount of total nitrogen in a plant and consequently its low transport to the reproductive organs. This poverty in proteins is presumably brought about by these sucking insects.

*Discussion.* According to Kraus and Kraybill [1918] plants well supplied with nitrogen and low in carbohydrates are generally highly vegetative. On the contrary, low nitrogen with relatively high carbohydrates, which means a higher C/N ratio, reduces the vegetative growth without a corresponding

TABLE III  
*Details of analysis of the entire, uninfested and infested plants, 1933*

Date of sample	Relative infestation of the plant region	Per-centage of moisture	Per-centage of dry matter	Percentages from samples as such of				
				Nitrogen	Proteins	Ash	Fibre	Fat
5 August	Uninfested leaves	81.2	18.8	0.633	3.956	3.57	1.82	1.29
	Uninfested stem	80.9	19.1	0.543	3.393	3.76	1.77	1.10
	Uninfested stem	83.7	16.3	0.295	1.468	2.05	7.45	0.16
18 August	Uninfested leaves	81.5	18.5	0.522	3.575	2.51	9.49	0.25
	Uninfested stem	79.7	20.3	0.556	3.350	3.53	9.02	1.07
	Uninfested stem	83.9	16.1	0.536	3.350	4.09	7.11	0.95
2 September	Uninfested leaves	82.9	17.1	0.517	3.337	1.64	7.52	0.17
	Uninfested stem	80.1	19.9	0.517	3.231	1.66	8.18	1.17
	Uninfested stem	79.1	20.9	0.581	3.693	3.90	1.97	1.18
20 September	Uninfested leaves	80.2	19.8	0.519	3.366	3.86	2.14	1.24
	Uninfested stem	76.5	23.5	0.235	1.468	1.37	9.55	0.32
	Uninfested stem	80.6	19.4	0.396	3.725	1.70	11.50	...
4 October	Uninfested leaves	81.5	18.5	0.673	4.206	...	...	...
	Uninfested stem	78.9	21.1	0.538	3.487	3.24	2.12	1.10
	Uninfested stem	86.3	13.7	0.633	3.956	3.80	2.58	1.08
23 October	Uninfested leaves	83.5	16.5	0.166	0.800	1.27	6.49	0.14
	Uninfested stem	80.9	19.1	0.562	1.037	1.57	3.03	0.16
	Uninfested stem	79.8	20.2	0.578	3.612	3.55	2.25	1.17
6 November	Uninfested leaves	76.1	23.9	0.215	1.343	3.16	2.46	1.03
	Uninfested stem	78.7	21.3	0.211	1.318	1.66	...	0.27
	Uninfested stem	78.5	21.5	0.548	3.425	4.79	2.29	1.30
29 November	Uninfested leaves	74.0	26.0	0.610	3.812	4.00	2.29	1.11
	Uninfested stem	80.7	19.3	0.223	1.362	...	...	0.25
	Uninfested stem	84.6	15.4	0.352	2.200	...	...	0.18
12 December	Uninfested leaves	87.0	13.0	0.478	2.987	0.932	...	...
	Uninfested stem	78.5	21.5	0.524	3.275	1.330	2.23	1.29
	Uninfested stem	77.6	22.4	0.574	3.487	4.211	2.03	1.03
29 November	Uninfested leaves	76.7	23.3	0.228	1.425	3.187	...	...
	Uninfested stem	75.6	24.4	0.239	1.463	1.600	...	...
	Uninfested stem	82.3	17.7	0.398	1.487	1.561	4.87	0.21
12 December	Uninfested leaves	83.2	16.8	0.375	2.343	1.164	1.24	0.72
	Uninfested stem	79.7	20.3	0.512	3.200	1.021	1.24	0.41
	Uninfested stem	78.5	21.5	0.608	3.800	4.25	2.00	0.80
12 December	Uninfested leaves	75.1	24.9	0.194	1.212	4.37	1.83	0.76
	Uninfested stem	79.8	20.2	0.218	1.362	1.47	...	0.16
	Uninfested stem	77.8	22.2	0.513	3.206	1.99	7.15	2.08
12 December	Uninfested leaves	85.0	15.0	0.324	2.025	1.44	2.00	0.34
	Uninfested stem	79.8	20.2	0.480	3.000	0.98	2.16	1.08
	Uninfested stem	79.2	20.8	0.534	3.650	3.94	2.41	0.92
12 December	Uninfested leaves	76.7	23.3	0.188	1.175	3.86	...	0.15
	Uninfested stem	80.0	20.0	0.198	1.237	1.53	...	...
	Uninfested stem	75.3	24.7	0.573	3.581	1.60	9.27	2.40
12 December	Uninfested leaves	89.6	10.4	0.372	2.325	0.65	4.89	0.61
	Uninfested stem	...	...	...	...	...	...	...
	Uninfested stem	...	...	...	...	...	...	...

TABLE IV  
*Statistical analysis of the data in Table III*

Constituents	Difference of means : high minus low infestation	Standard error of the difference	Values of 't'	Remarks
Moisture . . .	-1.18	0.270	4.37*	
Nitrogen (from September onwards)	+0.068	0.011	6.20*	
Ash—				
1. Before October	+0.462	0.091	5.133*	
2. From October onwards	-0.432	0.210	2.050	
Fat . . . .	-0.123	0.030	4.124*	
Carbohydrates .	+1.027	0.229	4.465*	

\* Highly significant

increase in fruiting. If nitrogen supply is too low in the tissues, fruit buds will not develop or, if developed, will shed.

Carbohydrate-nitrogen ratio varies with different plants. According to Woo [1919], quite the reverse results may be obtained with identical ratios in plants of different natures. Carbohydrates and nitrogen compounds fluctuate throughout the growing period. The fluctuation of the carbohydrates, however, is in the reverse order of the nitrogen compounds. Hooker [1922] suggests that a lowering of C/N ratio enhances the vegetative growth whereas a high ratio induces fruit formation. Dastur and Raut [1935] have suggested that C/N ratios are probably the effect rather than the cause of differential vegetative and reproductive growth.

Our investigations have shown that in the beginning of the season the amount of nitrogen is relatively higher in the leaves of the uninfested plants than in those infested with white-flies. Johnson [1934] also arrived at a similar conclusion with regard to the injury caused by leaf-hopper (*Empoasca fabae*) to the foliage of *Solanum tuberosum*. From September onwards the conditions are reversed which yield statistically significant differences. This reversion is brought about probably because a much higher percentage of nitrogen along with mineral ash and fat is transported from the vegetative to the reproductive organs in the healthy plants to help in boll formation. Migration of various constituents has already been established by other workers. There are two factors concerned in the reproductive phase of a plant: (i) formation or non-formation of bolls and (ii) their development or shedding. Mason and Maskell [1931] state that fertilization markedly increases the rate of uptake of phosphorus and total ash as well as of carbohydrates and nitrogen by the ovule. They further point out that removal of

growing bolls is followed by marked increase in the concentration of total ash, nitrogen and carbohydrates in the leaves and stem tissues. Such a condition has been noticed as a result of white-fly attack where the bolls are too few and, therefore, some of the constituents actually increase in the vegetative regions. Similar view is also expressed by Mason and Phillis [1934]. Thus leaf is the main distributing centre of sugars and of mineral food materials and gradually the bolls drain the vegetative plant of its food materials; if the leaves fall off the flowers and bolls are starved and finally shed. Kundrin [1929] also maintains that development of flowers requires an increased supply of nitrogen and ash and results in a migration of these constituents from the vegetative to generative organs. Gregory [1934] refers to migration of nitrogen and concludes that after reaching a maximum it begins to fall in the leaves and a rapid transference takes place from leaves to bolls.

Thus we conclude :

- (i) since the healthy plants on the whole produce more of total nitrogen than the infested ones, under exactly similar agricultural treatments this difference may be attributed to insect attack as a result of which the sap of the plant is drained off ;
- (ii) that the percentage of nitrogen and mineral ash transported from the vegetative to the reproductive organs is much higher in the uninfested plants, evidently because a healthy plant being rich in these materials is in a position to maintain a regular and abundant supply of nutrition for the reproductive organs. The attacked plant, on the other hand, having a meagre stock has to keep the reproductive organs in an almost starved condition ;
- (iii) that the adverse effects on the infested plants as seen in non-formation or dropping of floral buds, etc., may be the result of some dislocation in the carbohydrate-protein balance, which is affected by the insect attack.

These factors interacting may upset the normal vegetative and reproductive functioning of a plant. Further, they reduce the floral bud formation as well as increase shedding of flowers and bolls and thus result in low yield and poor development of lint and seed.

## 2. *Effect of attack on total dry weight of the parts of a plant above soil*

To make an accurate estimate of the relative output and difference between uninfested and infested plants, experiments were designed during 1932, 1934 and 1935 to determine the total dry matter produced by plants under varied intensities of white-fly attack. Cotton was grown in a big cage divided into three compartments, each  $16.5 \times 16.5 \times 8$  ft. In one of these compartments, plants were heavily infested artificially, in another a moderate attack was maintained while in the third the plants were kept under low infestation which meant that they were almost free from attack.

During 1932 and 1934 two sets of 10 plants each were under observation in each of these cages. The relative position of these sets of plants was similar in all the cases. A cloth catch-net was fixed under each set to collect the material shed by these plants. The material from each set was then

dried and weighed separately. At the end of the season, however, the weight of the stems, capsules, unshed leaves and the *kapas* (seed cotton) picked from the respective sets was similarly determined.

The white-fly infestation in these cages was relatively higher in 1932 than that in the other years. It is obvious from the data in Table V that, on an average, the total dry matter produced per plant varied with the intensity of attack, being the highest under least infestation and lowest under severe attack. During 1932, the uninfested plants produced, on an average, 352.6 gm. of dry matter per plant against 191.8 gm. produced by the infested plants. In 1934 the uninfested plants produced, on an average, 407.6 gm. of dry weight per plant against 315.1 gm. by the infested plants. The average for both the years indicates that the total dry matter produced by the uninfested and infested plants was 380.09 gm. and 253.47 gm. respectively. Thus, on an average, 49.9 per cent more of dry matter was produced by the uninfested plants.

An almost corresponding effect was noticed in shedding of leaves, floral buds and flowers in these plants. Relative shedding was 54.6 per cent in the uninfested and 55.9 per cent in the infested plants during 1932 and 42.0 per cent and 49.4 per cent respectively in 1934.

The effect of white-fly attack on the final yield was still more pronounced. During 1932, the average yield of *kapas* per plant varied from 58.1 gm. in the uninfested to 20.3 gm. in the infested plants, the difference being significant. In 1934, however, the corresponding yields were 98.9 gm. and 68.1 gm. respectively (Table VI) and the difference was still significant.

During 1935, the scheme of work was modified with a view to determine the rate of growth, of the plant, by noting its dry weight in different months, in relation to white-fly infestation.

Observations were made in two cages only. In each cage duplicate sets of six plants each were marked at corresponding places and catch-nets were fixed under individual sets. The plants in one of these cages were heavily infested with white-fly, while in the other cage they were kept almost free from attack.

Duplicate sets were removed every month from each cage during July to October, and the plants were chopped, dried and weighed as previously.

The data collected is presented in (Table VII).

Till the end of July the plants under observation grew almost equally in both the cages and produced, on an average, about 56.1 gm. of dry matter per plant. By the end of August, however, the infested plants produced 11.8 per cent less of dry weight per plant as compared with the uninfested ones. By the end of September again the infested plants showed 11.1 per cent less of dry weight. By the end of October, however, the infested plants produced 10.8 per cent less of dry matter in the vegetative region and 20.0 per cent less in the reproductive organs, as compared with the corresponding figures in the uninfested plants. It is, therefore, presumed that the reduction in the vegetative growth of a plant under white-fly attack is brought about during the month of August after which it continues almost at the same rate as in the uninfested plant, but the reproductive organs are seriously affected during the later period.

TABLE V

*Relative dry weights produced by the plants under different infestations of B. gossypiperda*

Treatment	Number of plants	Set No.	Average infestation per sq. in.	Total weight of shed material (gm.)	Total weight of sticks, leaves and capsules of the plants	Total weight of cotton picked (gm.)	Total dry weight produced (gm.)	Average dry weight per plant (gm.)	Percentage of shedding
1932									
Uninfested . . .	19	A	1.19	1452.20	1105.90	493.85	3051.95	} 352.61	54.59
		B	...	1602.85	1434.70	610.05	3847.60		
Moderately infested . . .	20	A	} 5.52	{ 1325.17	1142.60	551.95	3019.72	} 228.00	56.38
		B				795.41	498.00		
Infested . . .	20	A	} 11.12	{ 1133.88	964.00	232.40	2330.28	} 191.84	55.94
		B				784.99	547.20		
1934									
Uninfested . . .	20	A	} 0.32	{ 1436.1	1869.5	1001.6	4307.2	} 407.58	4.00
		B				1157.0	1711.3		
Moderately infested . . .	20	A	} 1.23	{ 1273.8	1212.1	693.0	3178.9	} 318.92	46.85
		B				1118.5	1501.0		
Infested . . .	20	A	} 7.50	{ 1277.8	1000.0	512.5	2790.3	} 315.10	49.41
		B				1163.8	1499.5		
Average									
Uninfested . . .	...	...	...	...	...	...	...	380.09	43.2)
Moderately infested . . .	...	...	...	...	...	...	...	273.46	51.62
Infested . . .	...	...	...	...	...	...	...	253.47	52.68
Mean . . .	...	...	...	...	...	...	...	302.34 ± 39.21	50.87 ± 1.2319

TABLE VI  
*Relative number of bolls and lint weight, etc. on plants under dry weight experiment, 1932 and 1934*

Year	Number of plants	Total number of bolls matured	Average number of bolls per plant	Total number of locks picked	Average number of locks per plant	Total weight of <i>lapas</i> (gm.)	Average weight per lock	Average weight of <i>lapas</i> per plant (gm.)
<i>1932</i>								
Uninfested . . .	19	366	19.3	1373	72.3	103.90	0.804	58.10
Moderately infested . .	20	305	15.3	1169	58.5	798.87	0.683	39.34
Infested . . .	20	169	8.5	657	32.9	406.70	0.618	20.34
Mean . . .	...	...	14.37 ± 3.152	...	...	...	0.702 ± 0.054	39.26 ± 10.90
<i>1934</i>								
Uninfested . . .	20	710	35.5	2759	137.9	1977.7	0.717	98.88
Moderately infested . .	20	565	28.2	2159	107.9	1273.1	0.589	63.15
Infested . . .	20	508	25.4	1961	98.0	1361.0	0.693	68.05
Mean . . .	...	...	29.70 ± 3.010	...	...	...	0.666 ± 0.039	76.69 ± 11.18

TABLE VII

*Relative dry weight produced by uninfested and infested plants, 1935*

Description of the set	Total number of plants examined	Total weight produced by the end of							Remarks
		July (gm.)	August (gm.)		September		October		
			Vegetative portion (gm.)	Reproductive portion (gm.)	Vegetative portion (gm.)	Reproductive portion (gm.)	Vegetative portion (gm.)	Reproductive portion (gm.)	
Uninfested—									* In September only one set of six plants was examined from each cage.
A . . . . .	6	408.0	943.0	1627.0	56.5	2650.1	786.8		
B . . . . .	6	263.0	942.0	...*	...*	2206.0	416.5		
Total . . . . .	12	673.0	1885.0	1627.0	56.5	4856.1	1203.3		
Average weight per plant . . . . .	...	56.1	157.1	271.2	9.4	404.7	100.3		
Infested—									
A . . . . .	6	470.0	919.5	1446.0	58.5	2226.0	606.9		
B . . . . .	6	205.0	743.5	...*	...*	2106.5	355.0		
Total . . . . .	12	675.0	1663.0	1446.0	58.5	4332.5	961.9		
Average weight per plant . . . . .	...	56.2	138.6	241.0	9.7	361.0	80.2		

• In September only one set of six plants was examined from each cage.

The above data show conclusively that the uninfested plants produce relatively more of total dry material than the infested plants, and are, therefore, better developed. Thus, the white-fly attack seriously interferes with the normal plant growth whereby the infested plants suffer both with respect to their vegetative and reproductive development. Unhampered vegetative growth in the healthy plants, on the other hand, encourages boll production and ultimately affects the yield favourably.

### 3. *Effect and after-effects of the attack*

The effect and after-effects of the white-fly attack on the vegetative growth of cotton plants and, subsequently, their capacity for producing flowers and bolls were investigated under various intensities of attack.

Experiments by Husain and Trehan [1933] were further modified and the various effects of severe infestation for a month's duration at different periods of plant growth were studied.

For these observations plants were grown under cages 6 ft.  $\times$  6 ft.  $\times$  6 ft. or 9 ft.  $\times$  9 ft.  $\times$  6 ft. Except the plants which were kept almost free from infestation or under moderate attack throughout the season, each set was exposed to a severe white-fly attack for the required period. Before and after this period, however, the plants were kept free as far as possible by 'hand picking' and occasional sprayings if necessary.

The plants grown under cages behaved slightly differently from those out in the fields, but the results obtained under almost identical conditions were comparable. The following sets were under observation each year from 1931 to 1936 :

*Practically free from attack.* Four to fifteen plants were under observation. The plants grew exceedingly well and were very bushy. The number of leaves and the height attained by the individual plants were invariably the maximum as compared with those of the other sets.

*Normal infestation during the season.* Observations were made on four to fourteen plants. An infestation approximating that in the field was maintained every year and at times the adults were 'hand picked' and removed if multiplication appeared excessive. Although attempts were made to keep the attack under control, it appeared slightly higher than that outside. The plants grew normally but some of the middle and bottom leaves turned black by September and ultimately became flaccid and drooping.

*Heavy infestation during July only.* Observations were made on four to thirteen plants. After a severe infestation for one month, the adults were removed in August and the plants thoroughly sprayed. Spraying was repeated after a few days to ensure complete freedom from attack. During the period of infestation, growth of the plants was hampered considerably. The leaves turned black and drooped down. When the attack was removed by 'hand picking' and spraying after the specified period, the plants recovered very well and almost regained their normal size and shape.

*Heavy infestation from 15 July to 15 August.* The number of plants under this treatment ranged from eight to twelve. Till about the third week of July the plants grew very well but after that they received a severe set-back. During the period of heavy infestation, the leaves turned black and most of

them drooped down, the growth was almost checked and the plants appeared sickly. During 1934 the plants were so badly affected that they could not recover even after the attack was removed.

*Heavy infestation during August only.* Four to eight plants were under observation during 1931, 1934 and 1935. The plants behaved very satisfactorily till about the first week of August after which they turned very sickly and most of the middle and bottom leaves showed the characteristic signs of damage. The growth was almost at stand still and some of the bottom leaves reddened and dried up prematurely and were ultimately shed.

*Severe attack from middle of August to middle of September.* These observations were carried out from 1932 onward and six to eight plants were put under this treatment every year. In the first year the attack was maintained from 7 August to 9 September, but in the subsequent years the infestation extended from 15 August to 15 September. In general, the plants attained a good growth before they were exposed to attack, but during infestation period they appeared sickly and the bottom leaves were shed comparatively very early.

*Severe attack throughout the season.* This set was maintained only in 1931. The plants grew though not normally since almost all the leaves had turned black and drooped (Plate XXXI, fig. 1). Premature shedding of leaves and floral buds was also a conspicuous feature.

i. *Effect of the attack on growth of a plant.* Series of observations were made on the extent of growth in plants under respective sets. Weekly measurements of the main stem of individual plants were recorded for about four months commencing from the 1st of July each year.

These observations showed that during the period of heavy attack the vegetative growth is checked considerably (Table VIII) and in certain cases it may be almost stopped. The leaves produced are relatively fewer in number and smaller in size than those on the uninfested plants. The floral buds turn yellow and drop off. Thus, during the period of attack, the growth is affected very badly in all respects. If the attack is removed during the growing period, the plants can recover and regain their growth, and in such cases the plants may even resume their normal size. The minimum growth was noticed when the plants were under heavy infestation from the middle of July to the end of August. Comparing the uninfested plants with (1) infested from 1 to 31 July and (2) moderately attacked sets which were common in all the three years, the increase in height of the main stem differed significantly in the former but approached quite near the significance in the latter case.

Further, the detrimental effect of the white-fly attack, as is manifested by arresting the vegetative growth, gets prominent about a week to 10 days after the infestation has commenced. Moreover, the same effect is continued for almost the same period even after the attack is removed. This effect is quite clear from the conspicuous bends which are seen in Fig. 1. Plants under least infestation or those where moderate infestation was maintained continued to grow normally.

ii. *After-effects of attack on flower and boll formation.* In nature, the white-fly attack on cotton, subsides from September onward whereas the boll formation starts from about the middle of August and continues right up to

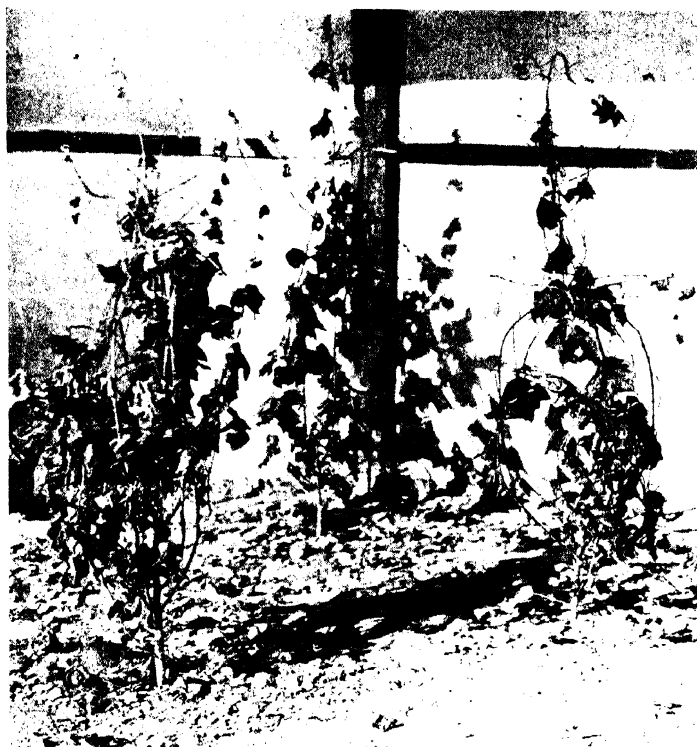


FIG. 1. Cotton plants under severe white-fly infestation

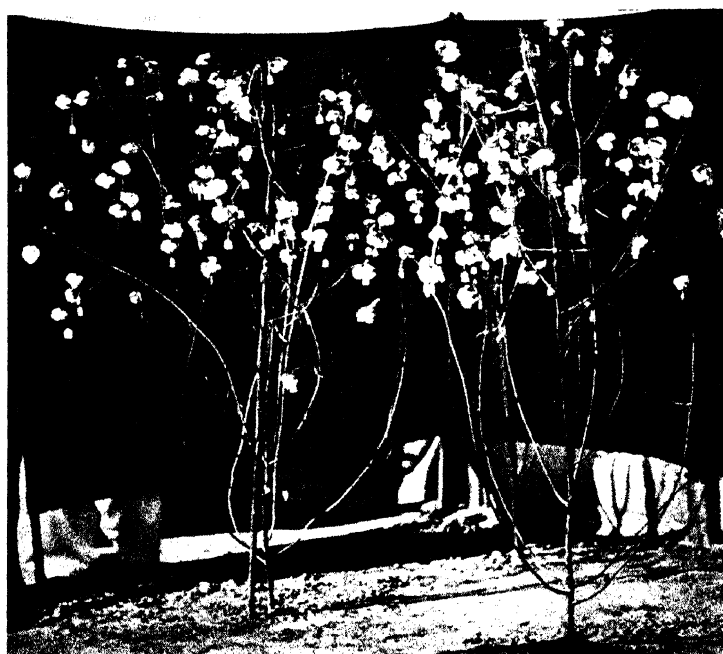


FIG. 2. Cotton plants practically free from white-fly infestation



TABLE VIII  
*Relative increase in height of plants under severe infestation of B. gossypiperda at different periods of their growth*

Description of plants	Average height of plants to start with	Average increase in height during					Total average height attained	Average increase in height	Remarks	
						ft. in.				
		July	August	September	October					
1931										
Uninfested . . . . .	1 8	9.8	10.8	17.3	3.3	5 1.2	3 5.2			
Moderately infested . . . . .	1 4	8.6	9.1	12.6	3.5	4 1.8	2 9.8			
Infested from 1 to 31 July . . . . .	2 2.5	2.7	4.8	7.5	5.5	3 11.0	1 8.5			
Infested from 1 to 31 August . . . . .	2 0.8	5.2	2.0	3.2	3.0	3 2.2	1 1.4			
1932										
Uninfested . . . . .	1 3	9.5	20.3	26.5	...	5 11.3	4 8.3		Observations upto September only	
Moderately infested . . . . .	2 0	8.5	10.5	19.3	...	5 2.3	3 2.3			
Infested from 1 to 31 July . . . . .	1 9	7.0	7.1	25.5	...	5 0.6	3 3.6			
Infested from 15 July to 15 August . . . . .	1 8	13.0	5.5	11.0	...	4 1.5	2 5.5			
Infested from 7 August to 9 September . . . . .	1 9	13.3	22.3	7.0	...	5 3.6	3 6.6			
1933										
Uninfested . . . . .	0 7.6	4.2	15.7	21.4	...	4 1.0	3 5.4		Under moderate infestation up to 5 August 1933 and least infestation after that	
Moderately infested . . . . .	1 7.2	3.0	7.3	15.0	...	3 8.5	2 1.3			
Infested from 1 to 31 July . . . . .	2 2.6	0.5	10.6	24.3	...	5 2.0	2 11.4			
Infested from 15 July to 15 August . . . . .	1 9.6	1.6	5.7	18.4	...	3 11.3	2 1.7			
Infested from 15 August to 15 September . . . . .	1 2.1	3.5	15.1	8.3	...	3 5.0	2 2.9			



Infested from 15 August to 15 September	1932	21-24	0-57	477	325	68-1	152	19-0	600	357	59-5	Eight plants Six plants Seven plants Six plants	Two died. Once sprayed by mistake. Infestation from August onwards. No flower appeared
	1933	15-19	0-88	128	84	63-3	47	7-8	92	79	85-8		
	1934	13-09	0-83	232	120	51-7	112	16-0	449	165	36-8		
	1935	12-40	0-59	315	199	63-1	116	19-3	448	67	15-0		
Average		15-57	0-66			60-97		18-10			37-10		
Infested severely throughout	1931	68-00	68-00	49	20	76-9	9	4-5			78-8	Four plants	Two died. Once sprayed by mistake. Infestation from August onwards. No flower appeared
	1932	27-87	27-87	3	3	100-0					...	Four plants	
	1935	20-00	29-00								...	Four plants	
Average		41-62	41-62										
S. E.						± 3-25		± 0-512			± 8-698		
Critical difference—						12-474		21-297			28-365		
5 per cent						15-148		30-897			41-269		
1 per cent													

N.B.—Analysis of the data for 1932, 1934 and 1935 only

the end of October. Does the white-fly leave a permanent effect on its host and if so, what are the results of such an after-effect? To ascertain these results, plants were artificially infested with the white-flies during different periods of their growth as previously described, and flower and boll formation recorded (Table IX and Fig. 2). Since all the treatments were not uniformly represented during 1931 and 1933, the data for 1932, 1934 and 1935 only were analysed statistically.

The following aspects of the problem were studied :

(a) *Shedding of flowers and bolls.* The lowest percentage shedding of flowers and bolls was noticed in the plants kept almost free from attack during the season. The plants heavily infested during the month of July showed an increased shedding of about 3 per cent and those under moderate attack 6.2 per cent, as compared with that of the uninfested plants. Similarly in the plants infested from 15 July to 15 August, and 15 August to 15 September the shedding was increased by 9.8 and 11.1 per cent respectively. Plants maintained under severe infestation throughout the season could not be compared because of their very poor condition in this respect.

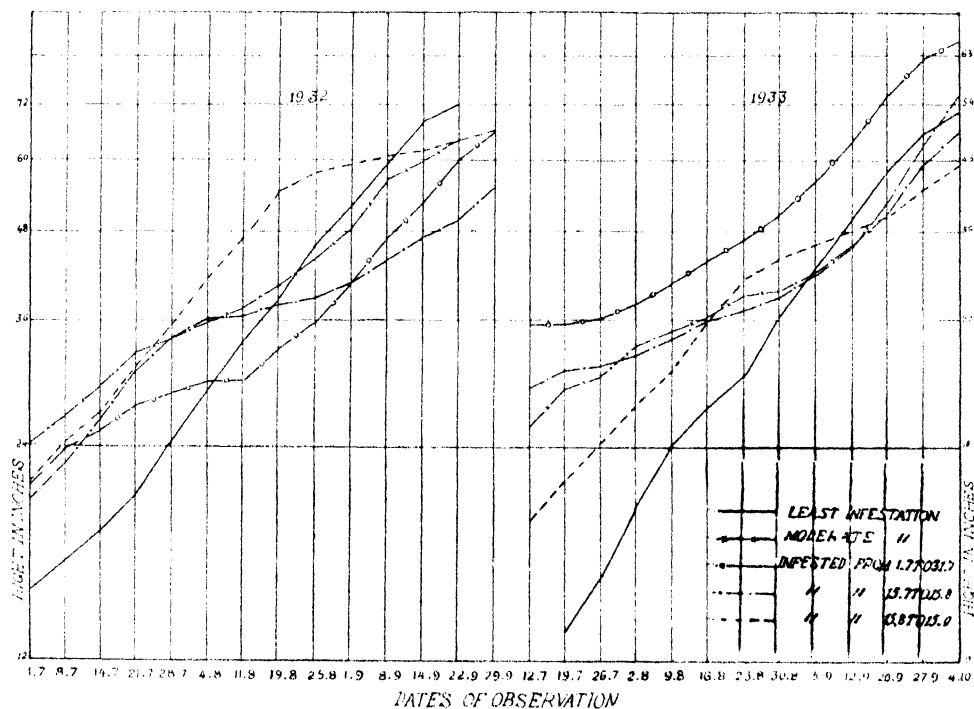


FIG. 1. Height of cotton plants under infestation of *B. gossypiperda* at different periods of growth

Thus, the percentage of shedding in flowers and bolls increased with the intensity of attack, varying, of course, with the period of infestation. Apart from the plants kept under least infestation, the minimum shedding was noticed in the plants infested only during July and maximum in those infested from the middle of August to middle of September. The differences, however, are not statistically significant.

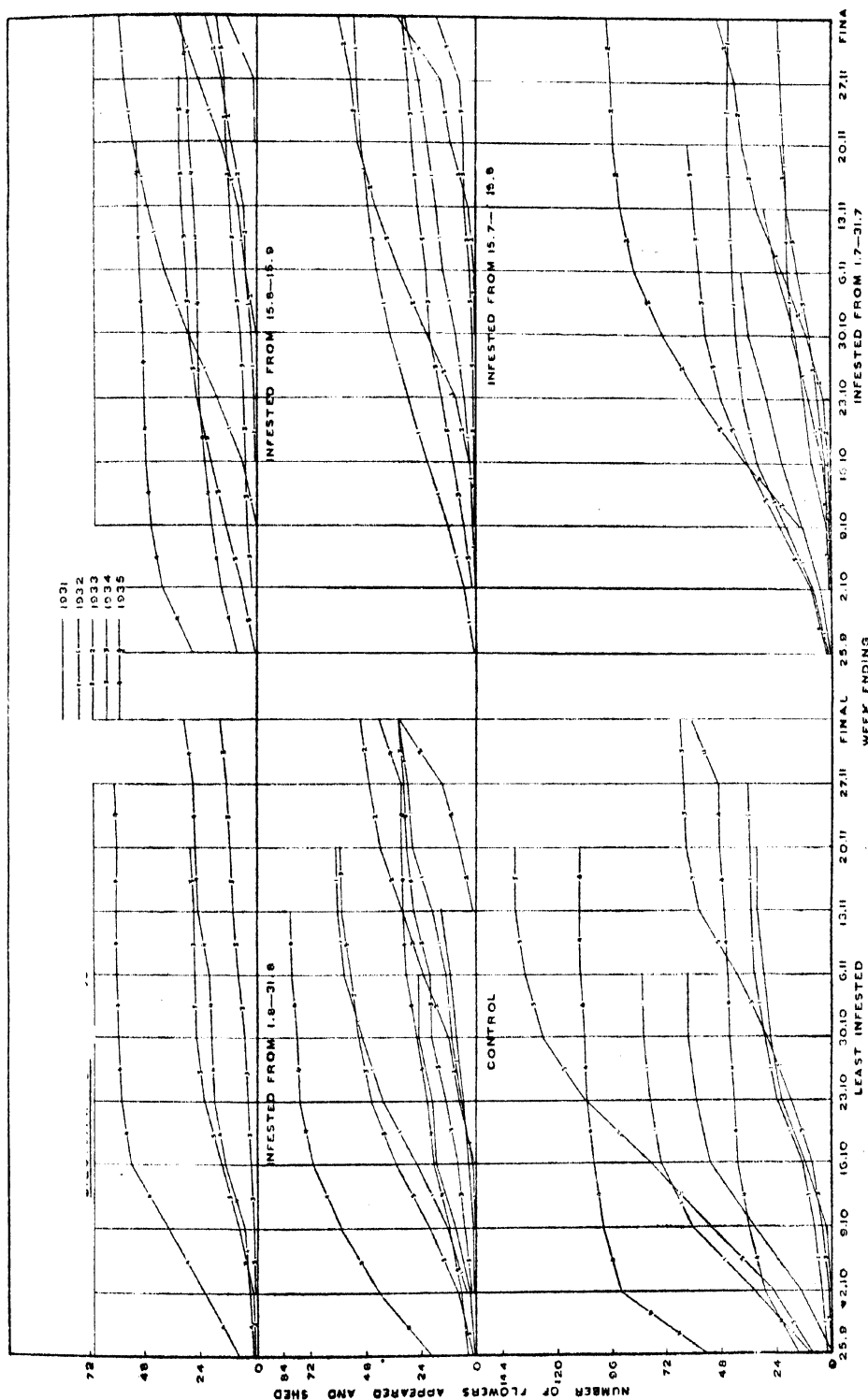


FIG. 2. Total No. of flowers appeared and shed per plant under different infestations of *B. gossypipenda* 1931-35

(b) *Number of bolls matured per plant.* The maximum number of bolls matured per plant was observed on the uninfested plants (Plate XXXI, fig. 2). When comparing the relative bearing on other sets with that on the uninfested plants, the number of bolls per plant, on an average, was 19.4, 23.2, 29.6 and 24.6 lower on those infested from 1 to 31 July, under moderate attack, 15 July to 15 August and 15 August to 15 September respectively. The statistical analysis indicates that the last three infestations have a significant effect on lowering the number of bolls per plant.

(c) *Bad opening of bolls.* Corresponding to the above results, the percentage of bad opening was almost in the reverse order. The lowest percentage of bad opening was observed in the bolls from the uninfested plants. The relative figures of all the above-stated sets of plants showed that the bad opening was higher by 13.4, 25.8, 41.0 and 21.1 per cent respectively as compared with that of the least infested plants and the difference between the uninfested and infested from 15 July to 15 August was statistically significant.

iii. *After-effects of attack on lint and seed development.* The data from these observations are given in Table X, with their statistical analysis in Table XI. Since all the treatments were not represented in 1931, the data for 1932 to 1935 were examined and the analysis of variance for the different characters is given in Table XI. The effect of treatments was significant for average lint per plant and average weight of lint per seed. On splitting up the four degrees of freedom for treatments it was found that the two groups, A and B (Table XI), differed significantly for all the characters given in the table. It may be pointed out that the plants infested from 1 July to 31 July which were included in group A behaved very near to the uninfested plants because infestation during the early period of growth did not produce any marked after-effects on the development and production of the plants when the attack was removed subsequently.

The mean variance for between groups was tested to the pooled error variance if the differences between the two components of error corresponding to between groups and remainder were non-significant. In the case of the average weight of lint per seed the error components differed significantly; hence the variance due to groups was tested against its own error. The variance for within A and B groups did not differ significantly. The following conclusions have been drawn:

i. *Average weight of kapas per plant.* The maximum production of kapas was obtained from the plants kept almost free from attack. The plants which suffered from a moderate attack produced only 44.4 per cent of the weight while those infested from 1 to 31 July, 15 July to 15 August and 15 August to 15 September produced 51.3, 31.2 and 27.7 per cent respectively as compared with the uninfested plants and the differences were highly significant between the two groups.

Since the development of bolls is judged by the condition of the locks, the average weight of kapas produced per plant was further calculated with respect to the number of locks. This showed that the locks of the uninfested plants and those of the plants infested during July only yielded relatively more of kapas per lock as compared with plants treated otherwise. Average weight of lint per lock also behaved correspondingly.

TABLE X  
Lint and seed record of the plants under different infestations of *B. gossypiperda* for the years 1932 to 1935

Treatment	Year	Total number of bolls picked	Total number of locks	Average number of locks per boll	Total weight of locks (gm.)	Average weight of lock (gm.)	Average weight of <i>kapas</i> per plant (gm.)	Total number of seeds	Average number of seeds per plant	Average number of seeds per boll	Average number of seed per lock	Total weight of lint (gm.)	Average lint per plant (gm.)	Average lint per boll (gm.)	Average lint per lock (gm.)	Average weight of lint per seed (gm.)	Average weight of single seed (gm.)	Average weight of 200 seeds (ave. range of 5 lots) (gm.)
Uninfested .	1932	187	748	4.000	412.20	0.55	103.00	4300	1073.0	23.0	5.74	120.20	324.30	0.61	0.172	0.030	0.005	12.87
	1933	85	316	3.719	204.89	0.65	51.22	1784	440.0	21.0	5.60	50.71	14.93	0.70	0.189	0.033	0.080	12.87
	1934	669	2563	3.831	883.30	0.34	28.33	9070	900.4	13.6	3.34	284.00	28.40	0.42	0.111	0.031	0.008	12.72
Average for 1932, 1933, 1934 and 1935		218	883	4.050	390.50	0.44	26.00	4491	290.4	20.6	5.09	120.00	8.60	0.59	0.146	0.028	0.036	11.80
						0.405	67.14		682.4			21.06			0.155	0.0305		13.57
Infested from 1 to 31 July	1932	169	667	3.946	331.90	0.49	41.5	3656	457.0	21.6	5.50	101.90	12.74	0.60	0.154	0.028	0.064	11.54
	1933	391	1576	4.030	605.33	0.61	120.9	8044	1188.0	22.0	5.65	280.15	30.22	0.74	0.183	0.032	0.073	14.42
	1934	303	1211	3.993	630.00	0.52	70.0	6835	746.1	22.4	5.60	186.00	20.07	0.61	0.153	0.027	0.064	13.66
	1935	143	541	3.857	271.50	0.50	50.9	3213	247.6	22.5	5.35	100.50	6.36	0.63	0.167	0.028	0.055	11.70
						0.530	63.33		647.2			130.15			0.164	0.0288		12.57
Infested from 15 July to 15 August	1932	159	621	3.905	280.72	0.46	30.21	3361	420.5	21.2	5.41	84.44	10.55	0.53	0.138	0.025	0.000	11.03
	1933	205	760	3.720	272.40	0.48	31.00	3065	458.1	17.9	5.10	78.58	9.82	0.38	0.109	0.021	0.051	10.59
	1934	116	462	3.982	171.00	0.37	21.40	2098	251.0	17.3	4.84	43.00	5.38	0.37	0.093	0.021	0.000	12.63
	1935	60	222	3.700	66.30	0.30	5.50	800	60.7	13.3	3.60	20.50	1.71	0.34	0.062	0.025	0.051	8.45
						0.378	24.30		290.1			6.87			0.108	0.023		10.68
Moderately infested	1932	111	456	4.000	108.00	0.37	42.00	2320	380.0	20.3	5.08	48.11	12.10	0.42	0.106	0.021	0.050	10.16
	1933	66	230	3.484	81.22	0.35	20.30	1065	260.2	16.1	4.63	23.53	5.88	0.35	0.102	0.022	0.051	10.32
	1934	86	341	3.965	150.00	0.38	32.50	1745	430.3	20.3	5.11	35.00	8.75	0.40	0.102	0.020	0.054	10.61
	1935	119	455	3.803	184.00	0.40	13.10	2316	165.4	19.5	5.11	58.50	4.17	0.49	0.129	0.025	0.052	10.62
						0.375	26.98		362.9			7.73			0.110	0.0220		10.43
Infested from 1 to 31 August	1934	109	432	3.903	157.00	0.31	17.10	1987	248.4	18.2	4.60	38.2	4.78	0.35	0.088	0.019	0.048	11.23
	1935	236	914	3.872	410.40	0.45	51.30	4396	540.5	18.6	4.81	129.4	16.17	0.55	0.141	0.029	0.062	12.58
Infested from 15 August to 15 September	1932	152	600	3.947	200.50	0.33	25.07	2834	354.3	18.6	4.63	56.88	7.11	0.37	0.097	0.020	0.049	9.63
	1933	30	92	3.066	20.80	0.23	3.47	323	80.8	10.8	3.51	5.41	1.35	0.18	0.059	0.016	0.041	8.46
	1934	112	449	4.000	156.00	0.31	19.86	2151	304.7	19.2	4.77	41.00	5.01	0.36	0.091	0.019	0.045	9.39
	1935	116	448	3.862	228.80	0.51	38.10	2188	364.6	18.8	4.88	60.90	10.15	0.52	0.136	0.027	0.073	13.24
						0.345	21.63		276.8			0.12			0.095	0.0205		10.68
S. E. Critical differences—					± 0.04737	± 13.176						± 3.051			± 0.001442		± 1.078	
5 per cent					0.1460	40.602						12.176			0.004444		3.8228	
1 per cent					0.2047	56.925						17.071			0.006230		4.6586	

TABLE XI  
Statistical analysis of the data in Table X

Sources of variation	D.F.	Average weight of kapas per plant			Average weight of kapas per lock			Average lint per plant			Average weight of lint per seed			Average weight of 200 seeds		
		S.S.	M.S.	F.	S.S.	M.S.	F.	S.S.	M.S.	F.	S.S.	M.S.	F.	S.S.	M.S.	F.
Total	19	19,153.5298	...	...	0.2275	...	...	1832.6112	...	...	0.0004329	...	...	92.7197	...	...
Years	3	2,691.8463	897.2821	...	0.0115	0.00383	...	234.4322	78.1441	...	0.0000229	0.00000763	...	3.1357	...	...
Treatments	4	8,128.0780	2,032.019	2.926	0.1083	0.0271	3.019	848.8248	212.2062	3.398	0.0003102	0.00007755	9.332	33.778	8.4446	1.816
Between groups (A and B)	1	8,041.7629	8,041.7629	11.5797†	0.1033	0.1033	11.510†	836.3521	836.3521	13.393	0.0002913	0.0002913	12.681	32.531	32.531	6.995
within A group	1	29.0700	29.0700	...	0.0024	0.0024	...	7.2962	7.2962	†	0.0000062	0.0000062	...	1.081	1.081	...
within B group	2	57.2450	28.6225	...	0.0026	0.0013	...	5.1763	2.5883	...	0.0000127	0.0000064	...	0.167	0.0835	...
Error	12	8,333.6055	694.4671	...	0.1077	0.008975	...	749.3542	62.4462	...	0.0000998	0.000008317	...	55.8056	4.6505	...
Error for between groups	3	2,624.8236	874.943	N.S.	0.0457	0.01523	N.S.	219.3746	73.1249	N.S.	0.0000689	0.00002297	S.	19.6235	6.5412	N.S.
	9	5,708.7789	634.308	...	0.0620	0.00689	...	529.9796	58.8868	...	0.0000309	0.00000343	...	36.1821	4.0202	...

N.B.—Group 'A' indicates plants uninfested and those infested for 1 July—31 July. Group 'B' indicates the remaining three sets taken together

\* Significant  
† Highly Significant  
‡ Significant  
N.S. Non Significant

ii. *Average number of seeds and weight of lint per plant.* Corresponding to the weight of *kapas*, the seed and lint per plant were also affected according to the intensity of attack and the period of infestation.

Further, seed weight was estimated from 1,000 seeds from each set in five lots of 200 each. The seeds from the uninfested plants, on the whole, yielded higher weight as compared with other sets.

It is, therefore, observed (Table XI) that the relative production of both these factors, namely seed and lint per plant and weight of seed, are seriously affected by the white-fly attack. Their relative values, however, indicate that the average weight of lint per seed is affected rather adversely and the differences are highly significant.

Results of considerable interest were obtained when the relative development of lint and seed per lock was compared with that of the control plants. In this way the actual production in various sets was judged on the basis of the expected one which was likely to be produced under normal conditions of attack as shown in Table XII. Thus, it was estimated that the bolls from the uninfested plants produced about 33.3 per cent more of lint and 25.7 per cent less of seed than what they were expected to produce under normal conditions. Similarly the lint produced by the plants heavily infested from 1 to 31 July, 15 July to 15 August and 1 to 31 August was 23.1 per cent, 2.5 per cent and 4.6 per cent higher respectively whereas it was lower when the infestation extended from 15 August to 15 September. The seed weight on the contrary showed the reverse order.

Since it has been observed that the white-fly attack is detrimental in all respects, it is evident that the cotton plant suffers more or less in all its phases. The above data, however, have shown that comparatively more damage is brought about if the attack appears in the latter part of the growing period. This obviously is within expectation, because during that critical period most important changes and adjustments in the vital nutrients take place in the plant tissue and any disturbance during the flowering period or a little before it is likely to act adversely on the subsequent yield.

*White-fly and the leaf curl of cotton and zinnia.* Mathur [1933] states that *B. gossypiperda* is responsible for producing leaf crinkle in zinnias at Dehra Dun. He further remarked that the leaf crinkle of cotton in the Sudan was identical with that in zinnia at Dehra Dun, the vector being the same.

There is no leaf crinkle of cotton in the Punjab, but a disease called the 'smalling disease' of cotton is common in certain localities of the province.

To study the rôle of *B. gossypiperda* in causing the so-called 'smalling disease' of cotton or the leaf curl in zinnias, some observations were made during 1932 and 1933 and the following scheme was adopted :

1. Adults of *B. gossypiperda* were collected from malformed leaves of zinnia and dwarf plants of cotton in nature and were sleeved on to healthy seedlings of cotton and on zinnia.

2. Adults of *B. gossypiperda* which emerged from the nymphs bred on malformed leaves of zinnia or on cotton plants suffering from 'smalling disease' were liberated on healthy seedlings of cotton and zinnia.

In both cases no crinkling was produced on zinnia and no smalling or crinkling on cotton. The results of these observations are given in Table XIII.

TABLE XII  
*Development of lint and seed under varied white-fly infestation, in relation to the production under moderate attack*

Sets under comparison	Weight of lint per boll (Average of all years)	Number of seeds per boll (Average of all years)	Lint expected on the basis of the number of seeds (gm.)	Difference between the actual production and the expected	Number of seeds expected on the basis of the observed lint weight	Difference between the actual production and the expected	Percentage of increase or decrease in the lint produced	Percentage of increase or decrease in the seeds produced
Uninfested	0.60	19.3	0.45	-0.15	26.0	-6.7	+33.3*	-25.7
Moderately infested	0.44	19.0						
Infested from 1 July to 31 July	0.64	22.4	0.52	-0.12	27.6	-5.2	+23.1	-18.8
Moderately infested	0.44	19.0						
Infested from 15 July to 15 August	0.41	17.4	0.40	-0.01	17.7	-0.3	+2.5	-1.7
Moderately infested	0.44	19.0						
Infested from 1 August to 31 August	0.45	18.4	0.43	-0.02	19.4	-1.0	+4.6	-5.1
Moderately infested	0.44	19.0						
Infested from 15 August to 15 September	0.36	16.9	0.39	-0.03	15.5	+1.4	-7.7	+9.0
Moderately infested	0.44	19.0						

+ Indicates increase

- Indicates decrease

N, B.—(i) The normal lint value is the average weight of lint per seed of the moderately infested =  $0.44 = 0.0232$  gm. and the expected value for other treatments are obtained by multiplying the number of seeds with this factor

(ii) The normal number of seeds is obtained from the moderately infested treatment corresponding to unit lint  $19.0 = \frac{1}{0.0232}$  and the corresponding expected number of seeds are obtained by multiplying the amount of lint with this factor

TABLE XIII

*Negative results in the transmission of leaf curl disease by B. gossypiperda in the Punjab*

Date	Number of adults liberated	Adults bred or collected from	Plants on which adults liberated	Number of leaves on the plant	New adults emerged on	Remarks
<i>1932</i>						
29 August	Numerous	Cotton plant with 'smalling'	Cotton	...	21 September 1932	No crinkling. Second generation also completed by 12 December 1932
21 September	16	Bred on cotton plant with 'smalling'	Do.	3	12 October 1932	No crinkling. New and the old leaves normal
	17	Do.	Do.	5	Do.	Do.
	23	Do.	Do.	4	Do.	Do.
	22	Do.	Do.	4	Do.	Do.
	15	Collected from zinnia	Do.	5	Do.	Do.
	13	Do.	Do.	4	Do.	Do.
	25	Do.	Do.	4	Do.	Do.
	Numerous	From those emerged on 21 September 1932	Do.	...	Do.	Very severe infestation of nymphs, etc., but no crinkling up to 12 October 1932
<i>1933</i>						
14 August	Numerous adults	On cotton plants in a cage	Zinnia	Four plants	...	The plants were kept in a cage where cotton plants were very severely infested by the white-fly. Zinnia plants were severely infested but no crinkling was observed till 8 October 1933. In one plant, however, the top leaves showed some malformation. New leaves were quite normal
11 November	7	Bred on cotton plant with 'smalling'	Do.	One plant with four leaves	...	By 15 October, the number of leaves increased to 12. When the sleeve was removed the smaller leaves were found a little crumpled
16 September	12	Do.	Cotton	3	...	The plant grew nicely upto 4 October and the leaves were quite normal. The plant died on 6 October 1933
29 September	8	Do.	Do.	3	...	Both the plants grew quite healthy when they were kept under observation till 14 November 1933
Do.	6	Do.	Do.	3	...	

The data presented above are meagre and it is not very safe to draw any conclusive statement. It may, however, be pointed out that *B. gossypiperda* has not given any indication of being the direct cause of producing the crinkling or the 'smalling' disease in zinnia or cotton respectively in the Punjab. Further, the abundance of the white-fly and corresponding absence

of any pathogenic disease even in zinnia plants which were enclosed in cages and thus exposed to severe infestation by this pest excludes all possibilities of its being the transmitter of such a disease. The plants under observation were kept in those cages for about 45 days, but symptoms of disease were not noticed. In one case only the top leaves showed a little malformation. This was probably the result of very high infestation and a consequent desapping of the foliage.

These investigations show that the causative organism of leaf crinkle of the Sudan may very likely be absent in the white-flies of this province or at least it is not so virulent here. This conclusion is supported by the views of Butler [1934] who states 'The leaf curl in the Sudan was like malaria requiring both the insect and the parasite. Position in the Punjab where white-fly is abundant and leaf curl rare is exactly the same as in England where anophales occurred but no malaria as there were no parasites'. Jackson [1934] suggests 'Leaf roll was a physiological effect not associated with any pest, though an insect might be attracted by the sap condition arising out of the physiological state of the plant.'

Some results of our cage experiments during 1931 and again in 1934 may elucidate our contention and support the views expressed above. In a few plants which were artificially kept almost free from white-fly attack during the season, 'smalling' of leaves and branches appeared on some of the branches whereas those under severe infestation were quite free from these symptoms.

This disease, however, has not been regarded of hereditary nature by Mohammad Afzal [1935].

It is, therefore, presumed, as already pointed out by Husain [1930], that *B. gossypiperda* may not be regarded, so far, as a vector for the transmission of this disease in cottons or even in zinnia in the Punjab. However, the problem should be taken up more seriously since there is every possibility of the introduction of such diseases in the province as this white-fly has been proved by other workers to be the carrier of the leaf-curl virus in other parts of the country.

### SUMMARY

The nature of damage by Aleurodidae has not been rightly understood so far. The present investigations, therefore, were undertaken to analyse the various aspects of this problem.

In the absence of any mechanical injury to the plant tissues, the effects of the white-fly attack were studied in relation to the physiological changes in the plant, its rate of growth and its reproductive activities.

The percentage of moisture is relatively higher in the uninfested plants with a corresponding increase of dry matter in the infested ones. Healthy plants show a lower C/N ratio—a condition that has been shown to stimulate the vegetative and reproductive growth of the plant.

Nitrogen is higher in the foliage of the uninfested cotton plants till the middle of August, after which it may rise in the foliage of the infested plants. However, it is significantly higher in the bolls of the uninfested plants than in those of the infested ones.

A much higher percentage of nitrogen, ash and fat is transported from the vegetative to the reproductive organs in the uninfested plants.

Reduction of bolls on the infested plants may be the result of some dislocation in the carbohydrate and protein balance.

The total dry matter produced by the uninfested plants as a result of their growth far exceeds that produced by the infested ones and, on an average, may extend to about 40 per cent. Thus the vegetative and reproductive growths are superior in the case of uninfested plants.

During the period of severe infestation, the vegetative growth is checked and in severe cases of attack may be almost stopped.

The boll formation increases as the intensity of attack decreases while the shedding and bad opening of the bolls correspond with the increase in attack. The bolls produced by the uninfested plants are well developed, and yield a maximum weight of *kapas*. The severity of infestation particularly when it appears late in the growing season lowers the yield of lint and affects the plant more adversely in all respects.

*B. gossypiperda* has not been found in any way responsible for the transmission of the 'smalling' disease in cottons or the leaf-curl in zinnia in the Punjab.

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# SAMPLING OF SUGARCANE FOR CHEMICAL ANALYSIS, II

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(With one text-figure)

IN the first paper on the subject, the authors [1937] presented data which could be used to determine the size of the sample of sugarcane which would give analytical figures lying within the limits of accuracy desired by the investigator. It was shown that for ordinary analysis of sugarcane juice, a sample consisting of ten stools picked at random from the field (1/7 of an acre in size) will give as accurate results as may be required in routine analysis. If, however, the results are to be expressed as percentages on cane, one must have an accurate estimate of the juice per cent. and for this purpose a sample consisting of 25 stools was recommended.

The above conclusions were arrived at from a study of the data pertaining to Co 318 grown at Lyallpur during the season 1935-36. It was hoped that later on it would be possible to examine these conclusions with reference to other varieties grown at different stations and to find out the extent to which data collected subsequently would bear out the conclusions already reported.

With the above objects in view, the investigation was extended to a number of varieties grown at different stations, and in order to study the seasonal effect, the canes at most of the places were analysed on different dates during the crushing season.

Further, it has been suggested that for any particular limit of accuracy desired, the size of the sampling unit will depend upon the degree of maturity of the cane, i.e., the more mature the cane, the less will be the number of canes or cane units required to make up the sampling unit. In other words keeping the size of the sampling units the same, the degree of accuracy obtainable will increase with advance in the crushing season. This suggestion also required examination.

The investigation, the results of which are presented in the present paper, was carried out during the season 1936-37 and relates to the sugarcane crop grown under zemindari conditions.

The different varieties of sugarcane selected were analysed three to four times during the crushing season, as shown in the following statement :

Locality	Varieties	Date of analysis
Lyallpur . . .	Co 223, Co 285, Co 312, Co 313	16 December 1936, 27 January 1937, 3 March 1937, 9 April 1937
Gurdaspur . . .	Co 213, Co 285, Co 300, Co 312, Co 331	30 November 1936, 26 January 1937, 12 March 1937
Montgomery . . .	Co 223, Co 285, Co 290, Co 312	9 November 1936, 28 January 1937, 13 March 1937 (Only Co 223)
Rawalpindi . . .	Katha, Co 285, Co 312 . . .	21 December 1936

*Size of the sample unit.* In the first paper of the series the size of the sample recommended was ten stools but as this recommendation was based on the analytical data relating to only one variety, it could not be stated definitely that it would apply to all the varieties commonly grown in the Punjab. It was considered likely that in the case of certain varieties a smaller-sized sample may do as well, while in the case of others, larger samples may be required. In the present investigation, therefore, the size of the sample unit was fixed at five stools, since if and when required sample series of bigger sizes could be easily constructed from this.

Since the publication of the first paper of this series Arceneaux *et al.* [1939] published the results of their investigation on the relation between the size of the sample and the experimental error in the analysis of juice and the determination of the yield of sugar in connection with their sugarcane variety trials. They reported and compared two sets of errors arrived at in two different ways. The figures for average brix and sucrose percentage in juice were obtained from six replicate samples of 3, 5, 10, 20 and 40 stalks. One set of errors was obtained by analysing independently the data pertaining to each one of the six sampling units in all the five sample series. The other set of errors, however, was derived by dividing the standard deviation of the lowest-sized sample series by  $\sqrt{n}$ , where  $n$  denotes the multiple of the lowest-size sample corresponding to the larger size for which the error was to be worked out. In this case the multiples used were 5/3, 10/3, 20/3, and 40/3. As a result of this investigation, the above authors concluded that although there was a consistent decrease in the standard error of the average difference with an increase in the number of stalks per sample, yet these figures for the observed decrease fell short of those for the theoretical values which were obtained on the assumption of complete randomness of variation between the three stalk samples. They explained the difference in the two sets of figures to be probably due to plot differences in their varietal test. The results obtained by Holmes and O'Neal [1939], on the other hand, showed a very close agreement between the two sets of errors worked out as described above.

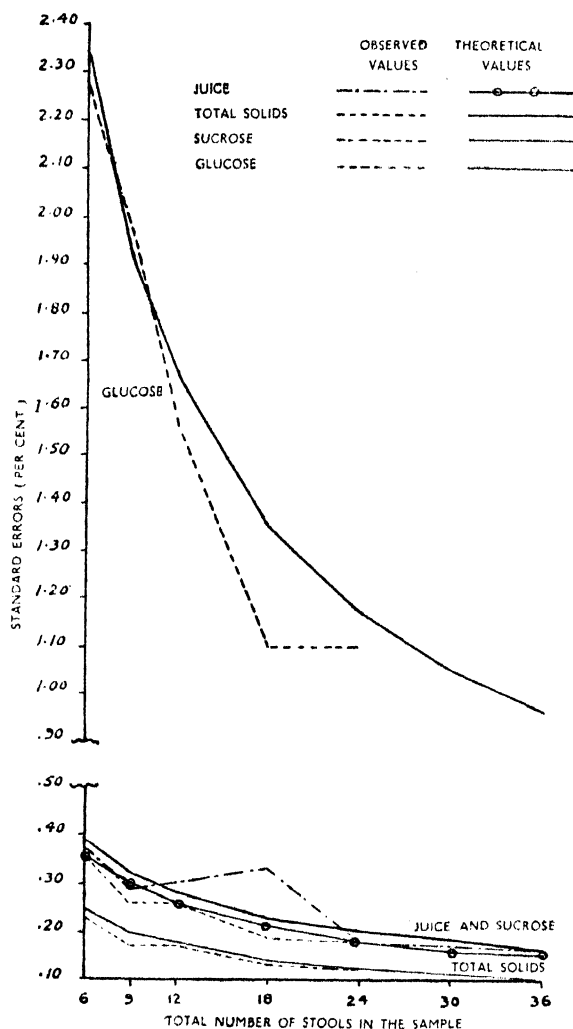


FIG. 1. Observed and theoretical standard errors per cent for Co 318, Lyallpur

As mentioned already, we had in view the construction of sample series of 10 or even 15 stool size from the lowest-sized sample series, viz., that of five stools but before selecting one of the two methods described above, it was considered desirable to examine them with reference to the data obtained at Lyallpur. Therefore, the values of the standard deviation for different constituents of cane for three stool sample series of Co 318 presented in Table IV of part I of the series [1936] have been used for calculating by the second method described above, the standard deviations for sample series consisting of sampling units of 6, 9, 12, 18, 24, 30 and 36 stools each. These values along with those already arrived at for the corresponding independently constructed sample series are given in Table I and represented

graphically in Fig. 1. It will be observed that, as observed by Holmes and O'Neal [1939], the two sets of values show an appreciable concordance.

TABLE I

*Relation between the standard deviations of the means for different constituent of sugarcane corresponding to sample units of different sizes (a) as determined independently for each sample series and (b) as calculated from the values for the lowest-sized sample series assuming complete randomness of variation within it*

Variety and size of the sample	Juice per cent		Total solids per cent		Sucrose per cent		Glucose per cent	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Co 318								
Lyallpur ( $n=48$ )								
3 stool samples . . .	0.320	...	0.070	...	0.084	...	0.012	...
6 Do. . .	0.240	0.226	0.046	0.050	0.054	0.059	0.008	0.009
9 Do. . .	0.182	0.185	0.034	0.040	0.040	0.049	0.007	0.007
12 Do. . .	0.189	0.160	0.034	0.035	0.040	0.042	0.006	0.006
18 Do. . .	0.205	0.131	0.025	0.029	0.029	0.034	0.004	0.00
24 Do. . .	0.110	0.113	0.024	0.025	0.027	0.030	0.004	0.004
30 Do. . .	0.108	0.101	...	0.022	...	0.027	...	0.004
36 Do. . .	0.100	0.092	...	0.020	...	0.024	...	0.003

Variety and size of the sample	Juice per cent		Total Solids per cent		Sucrose per cent	
	(a)	(b)	(a)	(b)	(a)	(b)
Katha						
Montgomery ( $n=16$ )						
1. Stool samples . . .	0.650	...	0.153	...	0.147	...
2. Do. . .	0.567	0.460	0.079	0.108	0.120	0.104
3. Do. . .	0.455	0.375	0.077	0.088	0.082	0.086
4. Do. . .	0.358	0.325	0.061	0.076	0.078	0.073
Co 285						
1. Stool samples . . .	0.366	...	0.160	...	0.202	...
2. Do. . .	0.180	0.259	0.099	0.113	0.133	0.143
3. Do. . .	0.189	0.211	0.080	0.092	0.099	0.117
4. Do. . .	0.119	0.183	0.058	0.080	0.058	0.101
Co 312						
1. Stool samples . . .	0.474	...	0.174	...	0.182	...
2. Do. . .	0.292	0.335	0.112	0.123	0.120	0.129
3. Do. . .	0.307	0.274	0.107	0.100	0.107	0.105
4. Do. . .	0.215	0.237	0.061	0.087	0.066	0.091

In the present study, therefore, we have worked out the standard deviations for ten stool samples by dividing the values of five-stool samples by  $\sqrt{2}$ .

As has already been stated, four varieties of sugarcane were analysed on four different dates at Lyallpur, five varieties on three different dates at Gurdaspur and three varieties on two different dates and also one on three different dates at Montgomery. At Rawalpindi, where, owing to the severity of winter, crushing of cane has to be finished before the onset of frost, the three varieties under observation were analysed only once.

As mentioned already, ten replicated samples of five stools each were obtained for the different varieties, the total area being about 1/16 acre under each variety with approximately 5 per cent samples on any one date of analysis. The chemical analysis included the determination of juice percentage, total solids and sucrose. Glucose was not estimated because the relative amount of this constituent present in the juice is very small and further, as has been shown in our previous paper, this figure being subject to the largest amount of error, any attempt to reduce it to the level of that for sucrose would be successful only if the sample size could be increased inordinately.

The analytical work carried out during the season 1936-37 at various places involved  $43 \times 10 = 430$  analyses, and as the presentation of the analytical data for so many samples of juice would have taken a large amount of space, these are not given and from the point of view of the problem in hand, the final figures of the coefficient of variation of the ten replicate samples of five stools each, determined separately for each variety and analysed on any particular date, have been considered sufficient.

Another important point is that during the year 1936-37 we were conducting a systematic sugarcane survey of some of the more important varieties growing in the districts of Lyallpur and Montgomery and had reserved for this enquiry about 10 *marlas* of a standing crop of average canes of each variety at various centres in these districts. For the sake of convenience and also with the object of forming an idea of the limits of accuracy in the collection and analysis of these sugarcane survey samples, work on the sampling of sugarcane was also carried out simultaneously on the same plots. Further, the sugarcane survey necessitated the withdrawal of large-sized samples every fortnight. It was, therefore, anticipated that as a result of repeated sampling and consequent thinning of the crop reserved, the errors in the replicate samples might be rather high. Some objection may be taken against the amalgamation of these two enquiries but in view of the fact that in a ripening test, samples of cane have to be withdrawn periodically from the same plots, it was thought that a study of the variations in the quality of the crop at definite intervals might prove to be of considerable interest.

The figures of the coefficients of variation mentioned above have been worked out for different constituents and the results presented in Table II. As will be seen, the data pertaining to any one variety analysed at different stations have, for the sake of convenience in presentation, been assembled together. The coefficients of variation have been worked out for juice and other constituents, both when these latter are expressed as percentages on juice and on cane.

TABLE II

*Coefficients of variation for different constituents of different varieties of sugarcane grown at various stations and analysed on different dates*

(Calculated from the data of the analyses of ten random samples of five stools each)  
(1936-37)

Variety	Station	Date of analysis	Constituents expressed on juice		Constituents expressed on cane		
			Total solids	Sucrose	Juice	Total solids	Sucrose
Co 285	Lyallpur	16-12-36	3.71	8.25	1.73	3.34	7.45
		27-1-37	2.00	5.50	3.71	4.03	6.83
		3-3-37	3.62	5.94	3.04	3.85	5.82
		9-4-37	3.90	6.09	3.89	4.48	6.37
		Mean	3.31	6.45	3.09	3.92	6.62
	Gurdaspur	30-11-36	4.25	5.83	5.73	3.23	4.00
		26-1-37	3.22	4.95	1.81	3.07	5.20
		12-3-37	2.51	3.34	5.75	5.86	6.22
		Mean	3.33	4.71	4.43	4.05	5.14
	Montgomery	9-11-36	2.68	3.98	1.95	1.49	2.63
		28-1-37	3.18	4.29	1.35	3.30	4.11
		Mean	2.93	4.13	1.65	2.40	3.37
	Rawalpindi	21-12-36	1.69	2.97	1.06	1.78	2.90
		Mean	3.08	5.11	3.00	3.44	5.15
Co 312	Lyallpur	16-12-36	5.85	8.96	1.44	6.34	9.85
		27-1-37	5.79	10.97	2.60	6.23	10.91
		3-3-37	4.76	6.11	3.16	7.59	8.28
		9-4-37	4.34	6.18	2.24	5.52	6.82
	Gurdaspur	Mean	5.19	8.05	2.36	6.42	8.96
		30-11-36	8.94	12.54	1.28	6.85	11.64
		26-1-37	5.57	10.81	0.68	6.04	11.29
		12-3-37	3.44	4.77	2.05	4.27	5.65
		Mean	5.98	9.37	1.34	5.72	9.53
	Montgomery	9-11-36	2.59	4.43	1.63	3.26	5.01
		28-1-37	6.09	10.02	2.78	7.42	10.31
		Mean	4.34	7.22	2.20	5.34	7.66
	Rawalpindi	21-12-36	1.46	3.12	1.12	2.07	3.49
		Mean	4.88	7.79	1.90	5.56	8.32

Variety	Station	Date of analysis	Constituents expressed on juice		Constituents expressed on cane		
			Total solids	Sucrose	Juice	Total solids	Sucrose
Co 223	Lyallpur .	16-12-36	2.77	3.86	2.24	2.25	3.31
"	"	27-1-37	3.18	5.50	2.04	3.21	4.95
"	"	3-3-37	3.04	4.86	2.83	2.75	3.83
"	"	9-4-37	4.06	5.98	3.56	5.78	6.91
		Mean .	3.26	5.05	2.67	3.50	4.75
"	Montgomery	9-11-36	5.14	7.22	2.45	4.71	6.96
"	"	28-1-37	1.81	3.67	1.58	2.01	3.86
"	"	13-3-37	1.18	1.88	1.27	1.29	1.78
		Mean .	2.71	4.26	1.76	2.61	4.20
	Mean .		3.03	4.71	2.28	3.14	4.51
Co 313	Lyallpur .	16-12-36	5.10	8.15	2.47	3.86	6.92
"	"	27-1-37	2.96	2.75	2.93	3.46	3.22
"	"	3-3-37	7.47	10.03	3.66	7.58	9.81
"	"	9-4-37	3.95	8.20	1.53	3.90	6.87
		Mean .	4.87	7.28	2.65	4.70	6.70
Co 213	Gurdaspur	30-11-36	5.72	7.95	1.56	5.08	7.27
"	"	26-1-37	3.55	5.65	1.13	3.48	5.61
"	"	12-3-37	5.63	5.43	1.92	4.46	5.70
		Mean .	4.97	6.34	1.54	4.34	6.19
Co 300	Gurdaspur	30-11-36	5.83	8.04	1.75	5.56	7.75
"	"	26-1-37	4.14	6.22	1.84	3.68	5.82
"	"	12-3-37	4.12	5.47	2.71	4.09	5.10
		Mean .	4.70	6.58	2.10	4.44	6.22
Co 331	Gurdaspur	30-11-36	6.45	10.27	2.23	5.98	9.40
"	"	26-1-37	3.03	5.63	2.31	4.94	7.48
"	"	12-3-37	6.82	9.28	2.80	6.75	8.48
		Mean .	5.43	8.39	2.45	5.89	8.45
Co 290	Montgomery	9-11-36	3.62	6.70	2.02	4.42	7.14
"	"	28-1-37	7.74	10.12	1.97	7.48	9.91
		Mean .	5.68	8.41	2.00	5.95	8.52
Katha	Rawalpindi	21-12-36	0.88	1.52	1.65	1.10	2.30
Co 318*	Lyallpur .	25-3-36	1.60	2.58	2.72	..	..

\*In this case instead of five stools, six stools constituted the sampling unit

The data presented in this table throw considerable light on the coefficient of variations for different constituents of cane as well as on the variations due to different varieties of cane, the influence of different stations, climatic and soil effects, and also the variations which occur at various stages of ripening of the cane during the period of its growth.

The results of the analysis of sugarcane are usually expressed either as percentages on juice or on cane. Whatever the method of expression adopted, the data presented in Table II show that the coefficient of variation is almost the same. This, however, is not in conformity with the findings relating to Co 285 and Katha reported in our previous paper. Those were based upon the data pertaining to only two varieties which were analysed once in the season. The conclusions now arrived at cover a much wider range for any variety with regard both to the dates of analyses and the localities surveyed and for this reason may be regarded as more reliable.

The coefficients of variation as affected by the different factors mentioned above will now be discussed separately under different heads.

#### *Coefficients of variation of the different constituents of cane*

Considering the mean values for the coefficients of variation for the three constituents, viz. juice, total solids and sucrose for all the varieties, it will be observed that, except in the case of Katha from Rawalpindi, this figure is the lowest for juice and highest for sucrose. The variability for sucrose is invariably higher than that for total solids, being about one and a half times the latter. This may be due to the fact that sucrose is more sensitive to changes in the environmental conditions than are total solids. It has been observed, for example, that, as a result of the action of frost upon the susceptible varieties of sugarcane, while total solids may not undergo any great change, the fall in the amount of sucrose due to inversion is relatively more pronounced.

#### *Variations between different varieties*

Considering the comparative figures of coefficients of variations of different varieties, it will be noticed that, as regards juice, Co 285 showed the highest variation both at Lyallpur and at Gurdaspur, while Co 312 at Gurdaspur gave the lowest figure. As far as total solids are concerned, the highest figure was obtained for Co 312 at Lyallpur. The values for this variety at Gurdaspur and Montgomery were also very high, being next only to Co 290 at Montgomery and Co 331 at Gurdaspur. Katha a local variety gave at Rawalpindi the lowest coefficient of variation for total solids and was followed by Co 285 and Co 223 at Montgomery. As regards sucrose, the highest figures were given by Co 312, Co 290 and Co 331 and lowest by Katha and Co 223, thus showing a high correlation between the variation in sucrose and that in total solids.

#### *Variations due to localities*

Since all the varieties were not available at different stations it is not possible to say precisely to what extent the meteorological and soil factors peculiar to different localities affect the extent of variation. However, the data relating to Co 285 and Co 312, both of which were available at three

stations, viz. Lyallpur, Gurdaspur and Montgomery and Co 313 from Lyallpur and Gurdaspur, supply a certain amount of information on the point. Considering the data for the two varieties collectively (Table III) the lowest figures for coefficients of variation both for juice and sucrose were obtained at Montgomery. This is rather surprising, since most of the land at this station is characterized by the presence of scattered patches containing a high concentration of salts, mostly those of sodium.

TABLE III

*Coefficients of variation for the same varieties in different localities*

	Lyallpur		Gurdaspur		Montgomery	
	Juice	Sucrose	Juice	Sucrose	Juice	Sucrose
Co 285 . . .	3.09	6.62	4.43	5.14	1.65	3.37
Co 312 . . .	2.36	8.96	1.34	9.53	2.20	7.66
Mean . . .	2.72	7.79	2.89	7.34	1.93	5.52
Co 313 . . .	2.65	6.70	1.54	6.19	..	..
Co 223 . . .	2.67	4.75	..	..	1.76	4.20

The corresponding figures from Lyallpur and Gurdaspur were almost the same. When, however, we consider the figures for Co 313 which was available both at Lyallpur and at Gurdaspur, we find that the coefficient of variation for juice was about 60 per cent higher at Lyallpur than at Gurdaspur which was also the case with Co 312. The figures for sucrose did not show any such difference. Evidently the data at our disposal are insufficient to warrant any general conclusions being drawn.

*Variations in relation to the degree of maturity of the crop*

Some workers believe that as the crop advances towards maturity the different constituents in cane reach a more stable figure. Therefore, late in the season, it may be possible to reduce the size of the sample without any appreciable sacrifice in accuracy. The data presented, however, do not lend support to such a view. In Table IV the figures for coefficient of variation of different varieties grown at various stations have been so re-arranged that the figures derived from analyses carried out early in the season appear in columns 1 and 2 and those relating to analyses done late in the season in columns 3 and 4. It will be seen that in a number of cases the variability is greater in the early season than in the late season, while in other cases quite the reverse is the case. The mean values for the two sets of variables given at the foot of the table do not differ appreciably from each other. This is easily explained if we take into consideration the fact that sugarcane is a very heterogeneous crop, and in a field, canes of different ages are available at any time during the season.

TABLE IV

*Coefficients of variation for different varieties of sugarcane as determined by analysing them early and late in the season*

				Analysed early in the season		Analysed late in the season	
				Juice	Sucrose	Juice	Sucrose
285	Lyallpur	.	.	1.73	7.45	3.47	6.10
„	Gurdaspur	.	.	5.73	4.00	5.75	6.22
„	Montgomery	.	.	1.95	2.63	1.35	4.11
312	Lyallpur	.	.	1.44	9.85	2.70	7.50
„	Gurdaspur	.	.	1.28	11.64	2.05	5.65
„	Montgomery	.	.	1.63	5.01	2.78	10.31
223	Lyallpur	.	.	2.24	3.31	3.20	5.37
„	Montgomery	.	.	2.45	6.96	1.27	1.78
313	Lyallpur	.	.	2.47	6.92	2.60	8.34
213	Gurdaspur	.	.	1.56	7.27	1.92	5.70
300	Gurdaspur	.	.	1.75	7.75	2.71	5.10
331	Gurdaspur	.	.	2.23	9.40	2.80	8.48
290	Montgomery	.	.	2.07	7.14	1.97	9.91
Mean				2.20	6.90	2.60	6.50

This point was further examined and more definite conclusions arrived at from the data relating to varietal trials carried out at Gurdaspur, Karnal and Montgomery during the season 1938-39. A number of varieties were analysed at these stations three times during the crushing season, viz. in December 1938 and January and February 1939. The system of replication followed at Gurdaspur was six varieties (Co 312, Co 313, Co 385, Co 421, Co 285 and Co 371) in four blocks; at Karnal there were six replications of five varieties (Co 312, Co 385, Co 421, Co 285 and Co 395) and at Montgomery three replications of five varieties (Co 312, Co 421, Co 285, Co 395 and Co 371). The size of the experimental plots at all the places was 1/40 of an acre and three sampling units of 10 stools each were taken for analysis from each plot. The above localities represent three important cane-growing tracts of the province which have different climatic conditions.

The data pertaining to the three dates of analysis have been examined only with reference to sucrose. The analyses of variance for each of the three dates have been worked out separately and are presented in Table V

TABLE V

*Analysis of variance of the values for sucrose estimated on three different dates*  
(Expressed as percentage on cane)

Station	Date of analysis	Source of variation	Degrees of freedom	Sum of squares	Mean square	Sampling error per sampling unit of 10 stools each	Mean value for sucrose	Coefficient of variation
Gurdaspur	December, 1938	Between plots	23	33.60	...			
		Within and between plots samples	48	6.32	0.132	$\pm 0.363$	7.96	4.56
Do.	January 1939	Between plots	23	47.51	...			
		Within and between plots samples	48	12.31	0.256	$\pm 0.506$	8.62	5.87
Do.	February, 1939	Between plots	23	67.10	...			
		Within and between plots samples	48	11.37	0.237	$\pm 0.487$	9.08	5.36
Karnal	December, 1938	Between plots	29	56.89	...			
		Within and between plots samples	60	7.11	0.118	$\pm 0.344$	9.38	3.67
Do.	January, 1939	Between Plots	29	77.76	...			
		Within and between plots samples	60	10.05	0.167	$\pm 0.411$	10.29	3.99
Do.	February, 1939	Between plots	29	68.51	...			
		Within and between plots samples	60	16.76	0.279	$\pm 0.528$	10.70	4.95
Montgomery	December, 1938	Between plots	14	38.04	...			
		Within and between plots samples	30	11.12	0.371	$\pm 0.609$	9.10	6.69
Do.	January, 1939	Between plots	14	24.15	...			
		Within and between plots samples	30	9.16	0.305	$\pm 0.552$	8.25	6.69
Do.	February, 1939	Between plots	14	39.36	...			
		Within and between plots samples	30	10.24	0.341	$\pm 0.584$	9.42	6.20

Considering the figures for sampling errors and the coefficients of variation for the three different dates it will be observed that there is no consistent difference between these three sets of figures at any of the stations. Besides the extent of differences between the figures for the three dates of analysis is not very pronounced at any station. These results confirm the conclusion already arrived at and show definitely that in order to obtain the same degree of accuracy in the figures of chemical analysis, one would not be justified in reducing the size of the sample when the canes are to be examined late in season.

TABLE VI  
*Mean values, standard deviations and coefficients of variation for juice, total solids and sucrose (on cane) in different varieties for five and ten stools samples*

Variety	Juice			Total solids						Sucrose					
	Per cent	Standard deviation		Coefficient of variation	Per cent	Standard deviation		Coefficient of variation	Per cent	Standard deviation		Coefficient of variation			
		5 stools	10 stools			5 stools	10 stools			5 stools	10 stools				
Co 285 .	63.3	1.90	1.34	3.00	2.12	11.10	0.38	0.27	3.44	2.43	8.99	0.46	0.33	5.15	3.64
Co 312 .	65.7	1.25	0.88	1.90	1.34	10.40	0.58	0.41	5.56	3.93	8.30	0.69	0.49	8.32	5.88
Co 223 .	65.7	1.50	1.06	2.28	1.61	11.51	0.36	0.25	3.14	2.22	9.69	0.44	0.31	4.51	3.19
Co 313 .	64.1	1.70	1.20	2.65	1.87	12.49	0.59	0.42	4.70	3.32	10.48	0.70	0.50	6.70	4.74
Co 213 .	67.3	1.04	0.74	1.54	1.09	10.60	0.46	0.33	4.34	3.07	8.53	0.53	0.37	6.19	4.38
Co 300 .	65.8	1.38	0.98	2.10	1.48	10.41	0.46	0.33	4.44	3.14	8.58	0.53	0.37	6.22	4.40
Co 331 .	64.9	1.59	1.12	2.45	1.73	10.01	0.59	0.42	5.89	4.16	7.76	0.66	0.47	8.45	5.98
Co 290 .	68.7	1.37	0.97	2.00	1.41	10.20	0.61	0.43	5.95	4.21	7.94	0.68	0.48	8.52	6.03
Mean .	65.7	1.47	1.04	2.24	1.58	10.84	0.50	0.36	4.68	3.31	8.79	0.59	0.42	6.76	4.78
Katha .	60.4	1.00	0.71	1.65	1.17	11.63	0.13	0.09	1.10	0.78	9.52	0.22	0.16	2.30	1.63
Co 318 .	62.5	1.66	1.29	2.66	2.06	12.49	0.20	0.15	1.60	1.24	9.60	0.24	0.19	2.50	1.94

*Limits of accuracy for samples of different sizes*

Most of the data discussed so far have been obtained from the examination of the results of five-stool samples and as such are not directly comparable with those given in the first paper of the series. For example, in that paper it was mentioned that the figures for sucrose and total solids expressed on juice were accurate within a range of about  $\pm 0.5$ , for glucose within  $\pm 0.10$  and for juice within  $\pm 2.5$ . In order to have an idea of the extent of agreement between these figures and those obtained now, the range of variations, for the mean values of juice, total solids and sucrose have been worked out and are given in Table VI. The standard deviations obtained are based upon the mean figures of the coefficients of variation for different varieties given in last line at the end of each variety in Table II.

The values for ten-stool samples are calculated from those for five-stool samples by the application of the formula  $\sigma - m = \frac{\sigma}{\sqrt{n}}$ . For the sake of comparison the values for Co 318 obtained previously have been recalculated so that they may become comparable with those for the other varieties now obtained and are given in the last line of the table in the column for five-stool samples. As a matter of fact, however, these are for six-stool samples. In the first paper of the series the limits of accuracy obtained finally were given in terms which could be compared directly with those obtained by Leather's method of representation [1913]. Therefore, to be strictly comparable to those obtained previously, the figures for limits of accuracy for sucrose given in Table VI should first be expressed on juice and then doubled.

Considering the figures for ten-stool samples, it will be observed that in the case of juice the range of accuracy for all the varieties with the exception of Co 285 is smaller than that obtained previously for Co 318. Even in the case of Co 285 it is only slightly wider. However, in the case of sucrose for all the varieties, except Katha, it is considerably higher than that for Co 318. The figures for Co 285, Co 223, Co 213, and Co 300 are almost double and for the remaining four varieties about two and a half times that of Co 318. But the figures now obtained, based as these are on a more comprehensive data, give a better estimate of the range of variation in accuracy for the varieties studied.

#### GENERAL CONCLUSIONS

As has been mentioned already, the present investigation was taken up with the object of determining how far the conclusions pertaining to the study of Co 318 grown at Lyallpur during 1935-36, reported in part I of the series were applicable to other varieties and also whether local conditions and seasonal variations modified the results to any appreciable extent.

It has been found that, of the coefficient of variations for the three constituents of cane, viz. juice, total solids and sucrose, the highest value has been obtained for sucrose and lowest for juice. This holds for all the varieties except Katha. Further, the range of variation in the values of the coefficient of variabilities is not the same for each variety. Differences in soil and climatic conditions may also exercise some influence in modifying these

values. However, the data examined do not show any wide differences in the values of the coefficients of variation when the canes are analysed during different periods in the season. The range of variation for some of the varieties analysed late in the season is as great as in the early season. Even for the same variety the coefficient of variability both in the early and in the late season may differ with locality. For example, the variabilities of the value of sucrose for Co 312 at Gurdaspur were found to be 11.64 and 5.65 respectively for the percentage of sucrose determined early and late in the season. The same variety at Montgomery, however, showed a variability of 5.01 in the early season as against 10.31 in the late season. Averaging out the effects of varieties and localities, we find that, as far as juice and sucrose are concerned, the variabilities found in the early season are not appreciably different from those in the late season. Confirmation of this conclusion has been obtained from the data for sucrose from varietal trials in which each variety was examined thrice in the season. These results, therefore, do not lend support to the view, which on theoretical considerations may seem to be plausible, that when a sample of sugarcane is to be analysed late in the season, one can reduce its size without any loss in accuracy.

As regards the diminution of error with increase in the size of the sample, it has been found that with a ten-stool sample, the error for the percentage of juice becomes as low as was obtained with Co 318. For sucrose, however, the errors are about two to two-and-half times as great. If, as recommended already, a ten-stool single sample is to be employed for chemical analysis, the mean errors attaching to juice, total solids and sucrose expressed as percentages on cane at 5 per cent level of probability will be  $\pm 2.1$ ,  $\pm 0.72$  and  $\pm 0.84$  respectively. These figures are evidently higher than those mentioned in the previous paper of the series, but based as they are on the data from eight varieties, these offer a wider range of application. Data relating to replicated varietal trials and the conclusions reached therefrom will be presented and discussed in a later contribution.

#### SUMMARY

The investigation reported in this paper was carried out in continuation of the work which has already been published as part I of the series.

Most of the data examined were obtained in connection with the sugarcane survey during the season 1936-37 and the results obtained were derived from and are applicable to the chemical analysis of sugarcane grown under zemindari conditions.

The values for the coefficients of variation for various constituents of cane discussed in this paper were arrived at from the figures for the lowest-sized sample by the use of the method which assumes a complete randomness of variation between the analytical values of different constituents in the case of the units comprising a five-stool sample. The values for the larger-sized samples thus calculated compare very favourably with those obtained from a random combination of original units. In dealing with the data from varietal trials, however, where a ten-stool sample was used, the coefficient of variation for sucrose was calculated directly.

The coefficients of variation of different constituents of sugarcane whether expressed as percentages on juice or on cane are almost the same. This conclusion, which is based upon the examination of a large number of cases and hence is more reliable, is at variance with that arrived at previously from a consideration of the data which related to only two varieties analysed once in the season.

A high correlation has been found to exist between the coefficients of variation for total solids and sucrose, the values for the former being invariably smaller than those for the latter.

Of the coefficients of variation for juice, total solids and sucrose, that for sucrose, in the case of all the varieties examined has been found to be the highest and for juice the lowest. The case of Katha, however, was an exception.

The different varieties examined do not follow the same order with regard to the coefficients of variation of their three constituents, viz. juice, total solids and sucrose.

The coefficient of variation associated with any definite size of the sample does not decrease with advance in season. Therefore, to obtain the same degree of accuracy late in the season, the size of the sample for chemical analysis cannot be reduced below that which is required early in the season.

The mean errors attaching to the estimation of juice, total solids and sucrose expressed on cane from a ten-stool sample have been found at the 5 per cent level of probability to be  $\pm 2.1$ ,  $\pm 0.72$  and  $\pm 0.84$  respectively for the above constituents. These figures are evidently different from those for Co 318 mentioned in the previous paper but based as they are on the data from eight different varieties these offer a wider range of application.

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# A DISEASE OF PIGEON-PEA [*CAJANUS CAJAN* (L.) MILLSP.] CAUSED BY *DIPLODIA CAJANI* SPEC. NOV.

BY

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(With Plate XXXII and four text-figures)

**D**ISEASED pigeon-pea [*Cajanus cajan* (L.) Millsp.] plants were received from the Botanical Sub-station, Imperial Agricultural Research Institute, Pusa, in November, 1939. On examination, the collar regions were found to be cankered. The cankers were of considerable size and deep seated, and were found to girdle the stem, leading ultimately to a collapse and death of the affected plants. The disease appeared to be different from those so far described on this crop from India.

Nowell [1923] reported three diseases of pigeon-pea in the West Indies, which infect roots, collar or lower stem. He stated that stem and collar-canker were observed in material from Carriacou and also referred to the existence of a serious stem-canker in Porto Rico, as recorded by Stevenson [1926].

Leach and Wright [1930] worked on the collar and stem-canker of (*Cajanus indicus*) pigeon-pea in Trinidad.

Recently Dastur [1939] reported that in the Central Provinces in 1938 well-grown cotton plants began to die off in large numbers. The stems were found to be broken a little above the ground level, and the owner of the crop attributed the death of the plants to a breaking of their backs. Dastur observed this breaking of the stem on *Phaseolus mungo* and *Cajanus indicus* to be accompanied by similar symptoms.

The work now presented was undertaken to determine the cause of the disease.

## SYMPTOMS OF THE DISEASE

The primary symptoms of the disease as studied from artificially infected plants are thickenings and distortions at the collar region. After 20 to 30 days, elliptical lesions of various sizes, with dark edges, are formed at the region of distortion. Later on, they are transformed into large, deep-seated cankers (Plate XXXII, fig. 1, *b*). In a few cases the diseased plants recover partially by the formation of callus but generally the cankers are found to spread and girdle the stem with the result that the plant collapses.

Very often the stem at the cankered portion presents a twisted appearance due to an unequal development of the wood. In advanced stages of the disease the internal tissues of the stem, a few inches above the canker, are also discoloured. No discoloration takes place in the root-system.

Adventitious roots develop just above the region of the canker (Plate XXXII, fig. 1, b).

When examined microscopically the infected tissue of the pigeon-pea is found to be slate-blue in colour as described by Leach and Wright [1930]. The mycelium is found to be present in the primary and secondary xylem vessels but there is no starch in the diseased tissues.

#### ISOLATION OF THE FUNGUS

Diseased pigeon-pea plants were obtained from Pusa in 1939. From five of these the cankered portions were separated. After surface-sterilization small bits of the infected tissue were immersed in one per cent silver nitrate for a minute, followed by dipping in one per cent sodium chloride. These pieces were then placed on potato-dextrose-agar in tubes. In about five to seven days fungal growth was observed on tissues taken from four out of the five plants. All the cultures appeared to be morphologically similar. On the tenth day pycnidia appeared in one culture and within another 15 days in all the others also. Single-spore cultures were started by the dilution plate method.

A similar disease again appeared on the pigeon-pea at Pusa in 1940. The fungus was isolated and appeared to be similar to the one obtained in the previous year. It was grown on potato-dextrose-agar. The isolates from the four diseased plants of 1939 crop were labelled A, C, D and E, and the one from the 1940 crop as D-47.

#### DETERMINATION OF PATHOGENICITY OF THE FUNGUS

For inoculation experiments, seeds of pigeon-pea (IP5) were first sterilized by dipping in formalin solution (1 : 320) for a minute and then dried by spreading them evenly on a sterilized petri-dish, kept covered with a piece of muslin for two hours. These seeds were sown in sterilized soil.

Pigeon-pea plants of different ages were inoculated separately with a pure culture of isolates A, C, D and E. The collar region of every seedling was cleaned with alcohol and then a piece of inoculum, removed from the edge of the plate culture, was placed upon it after or without wounding. A swab of damp and sterilized cotton-wool was then placed over every inoculated spot and wrapped with another thin piece of the same.

After inoculation every plant was covered with a glass case, the interior of which had been sprayed with water. All inoculated plants were kept in the pot-culture house for three days in order to protect them from outside injuries. The cotton-wool wrappers were removed from the collars after two weeks.

In all, four inoculation experiments were carried out with a view to ascertaining if the cultures were capable of producing the disease. The details of these experiments are set out in tabular form.

As culture No. D-47 was obtained as late as January, 1941 (from the pigeon-pea crop sown in 1940), it was not possible to make any inoculations with this isolate at the time of the first experiment.

In the following tables the sign ++++ represents typical symptoms associated with a very virulent attack by the pathogen; +++ typical symptoms with a fairly strong attack; ++ typical symptoms with a mild

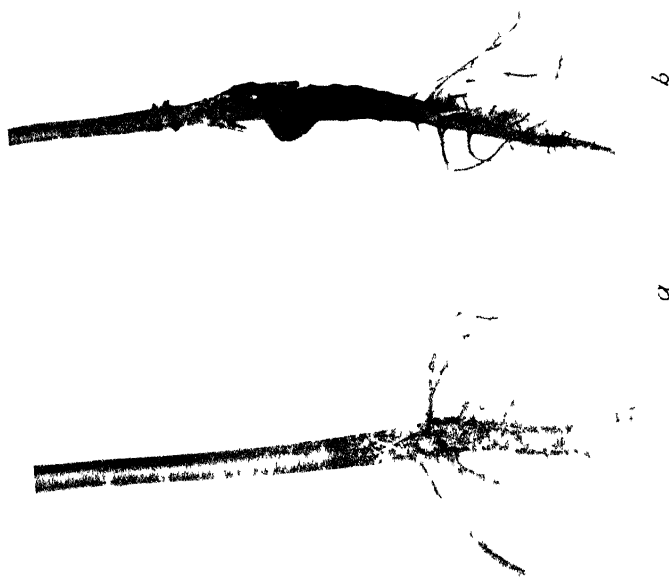


FIG 1 (a) *Cajanus cajan* plant used as un inoculated control  
(b) *C. cajan* plant inoculated with *D. platycephala*  
Raj Chaudhuri



FIG 2 Simple lobose pseudocyst of *Diplodia cajani* Raj Chaudhuri (1954)



FIG 3 Cyst of *Diplodia cajani* Raj Chaudhuri (1950)



attack ; + a little distortion and swelling at the collar region ; and — absence of infection.

Table I shows the results of the first experiment with seedlings  $2\frac{1}{2}$ —3 in. high.

TABLE I

*Inoculation of pigeon-peas with the fungus isolated from cankered plants*

(Inoculated on 30 January 1940 ; experiment discontinued after 30 July 1940)

Inoculum from isolate	Number of seedlings inoculated	Treatment		Type of infection		Death due to canker	
		After wounding	Without wounding	Wounded	Un-wounded	Wounded	Un-wounded
A . . . . .	10	5	5	+++	++	3	1
C . . . . .	10	5	5	++++	+++	5	4
D . . . . .	10	5	5	++++	+	0	0
E . . . . .	10	5	5	+	+	0	0
Control . . . . .	8	4	4	—	—	0	0

The plants in the first experiment were under observation for 181 days after which the experiment was discontinued. From the results given in Table I it is evident that isolate C was the most virulent in so far as all the plants inoculated with this isolate exhibited typical symptoms of canker ; all plants inoculated after wounding, and all but one not wounded died. The isolate A was fairly virulent pathogen, producing typical symptoms in all the inoculated plants and four out of ten died. Those inoculated with isolate D after wounding exhibited typical symptoms of the disease but very little swelling and distortion were observed in plants which were inoculated without wounding. None of the plants inoculated with this isolate died. Isolate E proved to be a weak pathogen and only a little distortion and swelling were produced at the collar. All the control plants were free from canker, and only one plant showed a small lesion on the wounded part, perhaps due to the healing effect.

In the second experiment two isolates, viz. C. and E were chosen to inoculate the seedlings  $2\frac{1}{2}$ —3 in. high. The results are shown in Table II.

TABLE II

*Inoculation of pigeon-peas with the isolates C and E*

(Inoculated on 27 April 1940 ; experiment discontinued after 9 August 1940)

Inoculum from isolate	Number of seedlings inoculated	Treatment		Type of infection		Death due to canker	
		After wounding	Without wounding	Wounded	Un-wounded	Wounded	Un-wounded
C . . . . .	40	20	20	++++	++	15	6
E . . . . .	40	20	20	+	+	0	0
Control . . . . .	40	20	20	—	—	0	0

The plants in the second experiment were under observation for 135 days, after which the experiment was discontinued. This experiment also proved that the isolate C was a very strong pathogen. The plants inoculated with this isolate exhibited typical symptoms of the disease. Seventy-five per cent of the plants inoculated after wounding died, and 30 per cent of those inoculated without wounding collapsed—altogether 21 plants out of 40 died due to the disease. Plants inoculated with isolate E showed a little swelling and distortion at the collar region, but none died. The control plants remained quite healthy and normal.

In the third experiment, 184 plants of two different ages were inoculated. The isolates used for inoculation were A and C. The results are given in Table III.

TABLE III

*Inoculation of pigeon-peas with isolates A and C*

(Inoculated on 23 August 1940; experiment discontinued after 3 December 1940)

Inoculum from isolates	Height of the seedlings (inches)	Age of seedlings (days)	Number of seedlings inoculated	Treatment		Type of infection		Death due to canker	
				After wounding	Without wounding	Wounded	Unwounded	Wounded	Unwounded
A . .	2½—3	9—10	40	20	20	+++	++	2	0
C . .	2½—3	10	40	20	20	+++	++	0	0
Control .	2½—3	9—11	40	20	20	—	—	0	0
A . .	5—6	21—22	22	11	11	+++	++	2	0
C . .	4—6	20—21	23	12	11	+++	++	0	0
Control .	5—6	20—22	19	10	9	—	—	0	0

The plants in the third experiment were under observation for 117 days, after which the experiment was discontinued. From Table III it is evident that the age of the plant makes practically no difference as far as the pathogenic activity of the fungus is concerned. Isolates A and C produced typical symptoms of the disease irrespective of the age of the host. As before, control plants were quite healthy and normal. Only a few plants died due to canker within the period of 117 days, possibly due to impaired activity on the part of the parasite owing to unfavourable weather conditions during the period of 23 August 1940 to 3 December 1940.

In the fourth experiment all the five isolates including D-47 (from diseased pigeon-pea plant of the 1940 crop) were used for inoculations. A large number of plants were inoculated with D-47 since this particular isolate was not used in the previous experiments. Results of the fourth experiment with seedlings 2½—3 inches high are given in Table IV.

TABLE IV

*Inoculation of pigeon-peas with isolates A, C, D, E and D-47*  
(Inoculated on 16 April 1941; experiment discontinued on 5 August 1941)

Inoculum from isolate	Number of seedlings inoculated	Treatment		Type of infection		Death due to canker	
		After wounding	Without wounding	Wounded	Unwounded	Wounded	Unwounded
A . . . . .	20	10	10	+++	++	6	1
C . . . . .	20	10	10	+++	++	9	3
D . . . . .	20	10	10	++	+	3	0
E . . . . .	20	10	10	+	+	1	0
D-47 . . . . .	80	40	40	++++	++	36	22
Control . . . . .	20	10	10	—	—	0	0

Table IV shows that isolate C and D-47 are very virulent.

It appears from the above experiments that plants which were inoculated after wounding suffered more from the disease than those which were inoculated without wounding, and that an injury at the collar region favours the development of the fungus as well as its pathogenic activity on the host.

The cankers produced in plants as a result of inoculations were identical with those occurring in nature and the discoloration of the internal tissues in the stem extended to a considerable distance. The fungus was also re-isolated from the cankers.

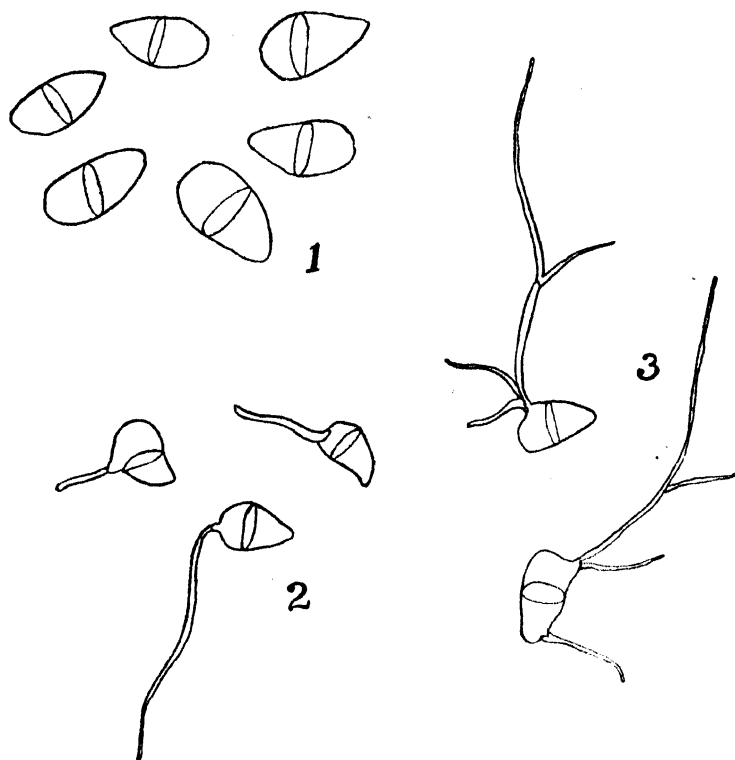
#### CHARACTERS OF THE PATHOGEN

The mycelium is septate, at first hyaline, but gradually becomes olive-green to brown, and ultimately, in mass, it appears black; its average width is  $4.3\mu$  with a range of  $2.6\mu$  to  $8.6\mu$ . There is abundant aerial mycelium.

The pycnidia are simple, globose (Plate XXXII, fig. 2), osteolate, immersed at first, later bursting through the epidermis, glabrous; the average diameter is  $405\mu$  with a range of  $301$ – $464\mu$ .

Conidia are borne on short needle-shaped conidiophores, at first hyaline, later turning from light to dark-brown; two-celled (Plate XXXII, fig. 3), mostly egg-shaped, sometimes ovoid to ellipsoid (Fig. 1), attached to the conidiophores with the narrower end; their average size is  $25.1 \times 12.7\mu$  with a range of  $21.5$ – $30.1 \times 10.8$ – $12.9\mu$ .

The upper cell of the conidium invariably germinates first. When a hanging drop culture was examined under the microscope it was found that a germ-tube is produced by the upper cell in about five hours (Fig. 2). The lower cell germinates after 15 to 20 hours in most cases. The germ-tube branches at the base, and sometimes the germ-tube produced by the upper cell branches considerably before that arising from the lower cell (Fig. 3).



FIGS. 1-3. *Diplodia cajani* n. sp. (1) conidia,  $\times 550$ ; (2) Germination of conidia,  $\times 430$ ; (3) Same, later stage,  $\times 430$

*Temperature relations.* The isolates A, C, D, E and D-47 were grown on plates of potato-dextrose-agar and their temperature curves are shown in Fig. 4.

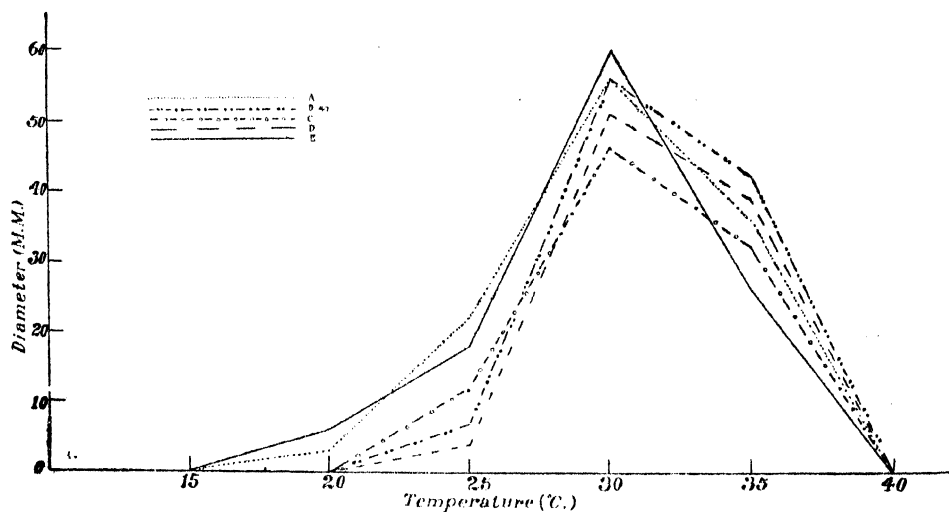


FIG. 4. Growth temperature curves of five isolates of *Diplodia cajani* n. sp. showing diameters of colonies after two weeks

The experiment was discontinued after two weeks. No growth was observed in any of the isolates at a temperature of 15°C. and below. A and E showed marked growth at 20°C. to 25°C., the respective diameters of the colonies being 3 mm. and 6 mm. at 20°C. and 22 mm. and 18 mm. at 25°C. Isolates C, D, and D-47 grew at 25°C. to 30°C. and their growth was found to be 12 mm., 4 mm. and 7 mm. at 25°C. and 46 mm., 51 mm. and 56 mm. at 30°C. respectively. In all cases maximum growth was found to take place at 30°C. and the growth of the isolate E was found to be 60 mm.

Above 30°C. the growth fell off rapidly with increasing temperature, so that the growth of A, C, D, E and D-47 at 35°C. was found to be 36 mm., 32 mm., 39 mm., 26 mm. and 42 mm. respectively and no growth was observed at 40°C.

An experiment was performed with a view to find out the influence of temperature on sporulation of each of the isolates when grown on different media.\* The fungus was grown on plain agar (two per cent), potato-dextrose-agar, oatmeal agar, Dox's agar and Brown's Standard Synthetic agar at six different temperatures, viz. 10°C., 15°C., 20°C., 25°C., 30°C. and 35°C. and the experiment was under observation for 76 days, after which it was discontinued.

All the isolates showed very poor growth and fruiting bodies were not formed on plain agar at 10°C., 15°C., 20°C., 25°C., 30°C. and 35°C. while most luxuriant growth accompanied by the best formation of pycnidia was observed on potato-dextrose-agar at all the temperatures mentioned above. Fair growth was observed on oatmeal agar at 10°C., while at higher temperatures the growth was very luxuriant. Pycnidia were produced only in one tube at 10°C. whereas at 20°C. and 25°C. pycnidia were developed in all the cultures on this medium. On Dox's agar luxuriant growth was not observed in all the cultures at 10°C. but at higher temperatures all the isolates showed vigorous growth, while pycnidia were regularly produced at 15°C., 20°C. (except in isolate E) and 25°C. On Brown's Standard Synthetic agar, growth appeared to be rather poor at 10°C. fair growth at 15°C. and 35°C. and luxuriant at 20°C., 25°C. and 30°C. Pycnidia were regularly developed only at 20°C. (except in the culture containing isolate C), and they were completely absent in all the cultures at 10°C., 25°C. and 35°C.

\* The following are the compositions of the media employed in the experiment, the quantities given being the amounts in one litre of the solution :—

- (1) Plain agar . . . . . Agar 20 gm.
- (2) Potato-dextrose-agar . . . . . Potato 200 gm., dextrose 20 gm., agar 20 gm.
- (3) Oatmeal agar . . . . . Oatmeal 30 gm., agar 20 gm.
- (4) Dox's agar . . . . . Magnesium sulphate 0.5 gm., potassium phosphate 1.0 gm., potassium chlorido 0.5 gm., ferrous sulphate 0.01 gm. (trace), sodium nitrate 2 gm., cane sugar 30 gm., bacto agar 15 gm.
- (5) Brown's Standard Synthetic agar . . . . . Glucose 2.0 gm., asparagin 2.0 gm., MgSO<sub>4</sub> 0.75 gm., K<sub>3</sub>PO<sub>4</sub> 1.24 gm., agar 15 gm.

Another experiment was performed with a view to determining the effect of different temperatures on pycnidial development on sterilized host tissues. The inoculated host tissues were subjected to three different temperatures, viz. 10°C., 20°C. and 30°C. and the experiment was under observation for 78 days, after which it was discontinued.

At 10°C. A and C exhibited very poor growth, D and E grew fairly well, while D-47 did not grow at all; pycnidia were produced only by D and E at this temperature.

At 20°C. luxuriant growth was observed in the case of A, D, E and D-47 while C exhibited fair growth. Pycnidia were produced in all the cultures at this temperature.

At 30°C. all the cultures exhibited luxuriant growth accompanied by regular development of pycnidia.

#### DISCUSSION

The fungus isolated from cankered pigeon-peas is a typical *Diplodia* capable of causing the disease under reference. Apart from the report of un-named species of *Diplodia* found by Leach and Wright [1930], the only other record of this genus on *Cajanus cajan* known to the author is that of *Diplodia cacaoicola* P. Henn. by Stevenson [1926]. The origin of this record is not known to the author, but it appears to be based on a conception of this species wide enough to include *Lasiodiplodia*, *Botryodiplodia* and *Chaetodiplodia*. Griffin and Moubiane had previously considered *D. cacaoicola* to be a *Lasiodiplodia*, which they called *L. Theobromae* (Syn., *Botryodiplodia Theobromae* Pat., *Macrophoma vestita* Prill. et Del., *Diplodia cacaoicola* P. Henn., and *Lasiodiplodia nigra* App. et Lambert). This inversion of genera is a clear indication of our poor understanding of morphological criteria in *Diplodia* and its related genera, and forces one back to the widely accepted practice of naming species on a basis of host relationship, coupled where possible with morphological criteria. It seems that the identity of the fungus on *Cajanus cajan* recorded by Stevenson was not based on the experimental study of the pathogen of the type of *Diplodia cacaoicola*, but on morphological criteria only.

*Diplodia dalbergiae* Died. was described by Sydow and Butler [1916] on *Dalbergia sisso* from Pulliyanur, Travancore, India. This member of the Leguminosae belongs to a tribe adjacent to that to which *Cajanus cajan* belongs, but apart from pathogenic considerations, the *Cajanus* fungus also differs from *Diplodia dalbergiae* also in having simple and not chambered pycnidia.

On the basis of a study of its pathogenicity and morphological characters, it is proposed to create a new species for the *Diplodia* causing canker on *Cajanus cajan*, and to name it *Diplodia cajani*.

*Diplodia cajani* spec. nov.

Pycnidia simple, globose, at first immersed, later erumpent, ostiolate, 405 (301-464) $\mu$ ; conidiophores needle-shaped; conidia at first continuous and hyaline, later one-septate and dark, upper cell rounded, lower cell tapering, 25.1  $\times$  12.7 (21.5-30.1  $\times$  10.8-12.9) $\mu$ .

*Habitat.* In living and dead stems of *Cajanus cajan* (L.) Millsp., Pusa, Bihar (October, 1939). Type in Herb. Crypt. Ind. Orient.; cultures in Type Culture Collection, Imperial Agricultural Research Institute, New Delhi.

*Latin diagnosis*

Pycnidiis uniloculatis, globosis, primo immersis, deinde erumpentibus, ostiolatis, 405 (301-464) $\mu$ ; conidiophoris acicularibus; sporulis primo continuis hyalinisque, deinde uniseptatis suscisque, cellula superiore rotundata, inferiore acuta,  $25.1 \times 12.7$  ( $21.5-30.1 \times 10.8-12.9$ ) $\mu$ .

*Habitat.* In ramis vivis et emortuis *Cajani cajan* (L.) Millsp., Pusa, Bihar (October, 1939). Typus in Herb. Crypt. Ind. Orient.; cultura in Collectione Culturarum Typicarum, Imperial Agricultural Research Institute, New Delhi.

Nowell [1933] reported stem and collar-canker of pigeon-pea. He found an ascomycete to be uniformly present in the diseased material. The fungus consisted of dark hyphae which gave a slaty appearance to the wood; a black stroma was produced throughout the bark with long naked perithecia which were produced in dense clusters and more or less united at the base. White tendrils of unicellular spores were ejected both from these and from adjacent pycnidia, the former being coffin-shaped, the latter oval or oblong. The perfect stage of the fungus was never obtained during the present study. Nowell reported that infection experiments were carried out in dry weather, and the results obtained were negative but in the present work all the four inoculation experiments proved to be successful and the pathogenicity of the fungus has been definitely established.

Leach and Wright [1930] isolated the following fungi from the cankered pigeon-pea plants :—

- (1) An Ascomycete with two pycnidial stages of *Phoma* and *Macrophoma* types
- (2) A *Cephalosporium*
- (3) Two species of *Fusarium*
- (4) Two species of *Diplodia*
- (5) *Myxosporium*

They found that the Ascomycete and one species of *Diplodia* were capable of producing infection. They inoculated pigeon-pea plants with the Ascomycete and a species of *Diplodia* and both were found to be capable of producing the infection. They inoculated pigeon-pea plants with the Ascomycete and *Diplodia* sp. at the stem, branch and collar regions, and found that the attack was most virulent at the collar region. The Ascomycete was capable of producing the infection in all the three regions, whereas the *Diplodia* sp. produced infection only at the collar region. It is very surprising to note that although the *Diplodia* sp. was more virulent at the collar region than the Ascomycete, and the number of plants cankered were more in the case of those inoculated with *Diplodia* sp. at the collar region, they write, the causal parasite of the disease, therefore, is an Ascomycete and it seems to be most virulent at the collar region. They state that the Ascomycete is a member of the genus *Physalospora*.

Dastur [1939], while working on the 'stem breaking' of cotton, isolated *Fusarium* spp., *Rhizoctonia bataticola* and *Colletotrichum* sp. from the broken parts of the stem. He found these fungi to be confined only to the dead tissues, and no hyphae were found in the living tissues. The bending and

breaking of the stem was considered to be due to high winds. He observed a similar disease on pigeon-peas. It is clear that the disease is distinct from the type of injury described by Dastur.

The four inoculation experiments definitely proved that although the fungus was capable of producing infection on the unwounded plants, an injury, in all the cases, enhanced the pathogenic activity of the fungus to a great extent.

While studying the cultural characteristics of the new fungus *Diplodia cajani* on various media, it was found that potato-dextrose-agar, oatmeal-agar, Dox's agar, Brown's Standard Synthetic agar and sterilized host tissue produced pycnidia in great abundance. The fungus, however, exhibited very poor growth on plain agar (two per cent) with the result that pycnidia were not produced.

Temperature relations of *Diplodia cajani* were determined by growing the fungus on plates of potato-dextrose-agar for a period of two weeks. At 30°C. the colonies reached maximum diameter, and this was found to be 60 mm. in the case of isolate E (Fig. 4). Next in order were A and D-47 which exhibited the colonies of same diameter measuring 56 mm.; while the colonies reached a diameter of 51 mm. and 46 mm. in the case of D and C respectively.

The growth fell off rapidly with increase of temperature, and at 35°C. the colonies showed a diameter of 26 mm., 36 mm., 42 mm., 39 mm., and 32 mm. in the case of E, A, D-47, D and C respectively, and the growth was altogether stopped at 40°C.

Below 30°C. the growth decreased rapidly with a decrease in temperature, and at 25°C. only 18 mm., 22 mm., 7 mm., 4 mm. and 12 mm. occurred in the case of E, A, D-47, D and C respectively. The growth of D-47, D and C stopped at a temperature of 20°C. while the isolates E and A showed no growth at 15°C.

Hence, it appears that the optimum temperature for the growth of the organism on potato-dextrose-agar was about 30°C. while the minimum and maximum lie somewhere below 20°C. and above 35°C. respectively.

Further, it was observed that of the various media tested potato-dextrose-agar appeared to be most suitable for the fungus, since very luxuriant growth accompanied by the production of pycnidia was found on this medium in all the temperatures ranging from 10°C. to 35°C. It was also found that the optimum temperature for the sporulation of the organism on the various media was 20°C.

The fungus was also grown on sterilized host tissues, and it was found that all the isolates grew vigorously and produced pycnidia in abundance at 20°C. and 30°C.

Leach and Wright [1930] stated that cankers, though they may be formed on apparently sound tissue, arise most commonly at points of injury caused by hoeing operations, breakages, and insects. Hence, they suggested that injuries at the collar regions caused during cultural operations and careless hoeing should always be avoided. The disease assumes its most serious aspect in this region, and care should be taken during hoeing operations. During the course of inoculation experiments it was found that the pigeon-pea plants which were injured at the collar region suffered the most virulent

attack of the pathogen, and hence it appears that damage due to the disease could be minimized to a great extent if necessary precautions are taken to avoid injuries at the collar region.

#### SUMMARY

Thickening and distortion at the collar region are the primary symptoms of the canker disease of pigeon-pea (*Cajanus cajan*). Later on lesions are formed at this region which are ultimately transformed into large deep-seated cankers. Very often adventitious roots develop in the neighbourhood of the cankered region.

Diseased pigeon-pea plants of the 1939 and 1940 crops were obtained from Pusa. The fungus isolated was in all cases a species of *Diplodia*.

Healthy pigeon-pea plants of different ages were inoculated with pure cultures of *Diplodia* at the collar region after wounding and without it. Several isolates produced typical canker and caused death of the inoculated plants.

The fungus was grown on plates of potato-dextrose-agar for a period of two weeks and the temperature relations were determined. No growth was observed at 15°C. or below it, and maximum growth took place at 30°C. above which it fell off rapidly with increasing temperature, and no growth was observed at 40°C.

It was found that potato-dextrose-agar was the most suitable medium for growth and sporulation of the fungus at all temperatures between 10°C. and 35°C. After a period of 76 days it was found that 20°C. was the optimum temperature for the sporulation of the fungus on the various media tested.

The fungus was also grown on sterilized host tissue at different temperatures, and after a period of 78 days it was found that 20°C. to 30°C. was the optimum temperature for growth and sporulation of the fungus.

Since the characteristics of this fungus did not agree with any known species of *Diplodia*, a new species has been created and named *Diplodia cajani*.

It was found that the attack of the pathogen was very virulent when the collar region was wounded before inoculation; hence it appears that damage due to the disease can be minimized to a great extent if injuries at the collar regions are avoided.

#### ACKNOWLEDGEMENTS

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# UTILIZATION OF WASTE PRODUCTS OF THE SUGAR INDUSTRY IN THE CANE FIELDS

## II. PREPARATION OF COMPOSTS BY HOT FERMENTATION

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**I**N Part I [Srivastava, Chaturvedi and Rao, 1940], attention was drawn to the large quantities of press-mud, cane trash and bagasse available in sugar factories which are not being utilized at present. Experiments were, therefore, started to determine the best method of composting these materials with a view to using them in the cane fields. Composts were prepared by the usual methods developed at Indore, i.e. by aerobic fermentation and also by an adapted compost process as recommended by Fowler. The resulting manure was quite satisfactory, but the process employed was too costly since periodical turning had to be given which required a lot of labour. There was also considerable loss of nitrogen varying from 20 to 50 per cent and of dry matter from 40 to 60 per cent depending on the initial composition of the heap. Though these losses were not abnormal when the nature of the process was taken into consideration, it was very desirable to reduce these, if possible.

Acharya and co-workers [1939] have claimed very good results for methods of composting by what they term hot fermentation, so far as conservation of nitrogen and dry matter is concerned. By this method, the material is subjected first for a short period to aerobic and then to anaerobic fermentation until the compost is ready. Experiments have now been carried out on the composting of the waste products of the sugar factory by hot fermentation and the results have been very encouraging.

### EXPERIMENTAL

*Method of composting.* For the preparation of the heaps, air-dried press-mud (moisture per cent 5.8), cane trash cut into lengths of two to three inches (moisture per cent 7.0) and air-dried bagasse (moisture per cent 4.2) were mixed in suitable proportions and well turned with a thin slurry of cowdung and molasses. The proportions used were such that nitrogen per cent of the heap was in the neighbourhood of 1.0 and C : N ratio, 30 : 1, for which purpose a 3 : 1 ratio of press-mud to bagasse or cane trash was most satisfactory. On the weight of the heap which was 600 lb., molasses and cowdung used were 2 per cent each (except in experiments 1A and 1B when the quantity was 3.3 per cent).

No turning was given to any heap, but care was taken to see that the heaps remained moist by sprinkling water whenever necessary. Composts were taken to be ready when the heaps had developed a crumbled powdery structure and a greyish-black appearance.

*Group I.* Experiments 1—3. The heaps were placed in trenches 6 ft.  $\times$  4 ft.  $\times$  3 ft. loosely packed so that aerobic fermentation could proceed. After exposure for seven to eight days, the heaps were covered with mud paste thus stopping aeration. Only anaerobic fermentation was then possible and this continued until the composts were ready.

*Group II.* Experiments 4—6. The heaps were placed in trenches and subjected to aerobic fermentation for about a week as in the previous case. The heaps were fairly closely packed later so that fermentation was practically anaerobic.

*Group III.* Experiments 7—9. Heaps were prepared on the ground and not in trenches. Otherwise, the procedure was the same as in group I.

*Group IV.* Experiments 10—12. Heaps were prepared on the ground and the procedure was as in group II.

Only sulphitation press-mud was used in all these experiments. Press-mud of carbonatation factories contains only 7 per cent organic matter expressed as C and 0.6 per cent N on a dry basis. Attempts were made to prepare composts from this material also by aerobic fermentation, but these did not prove satisfactory on account of the large amount of inorganic matter present. The small percentage of nitrogen—it is less than half that in sulphitation press-mud—precludes the addition of large quantities of organic matter in order to make up the deficiency.

TABLE I

Experiment	Composition of the heap					Time of com- posting	Per cent loss of dry matter	N per cent in the compost (on dry basis)	Per cent loss of nitrogen	
	Press- mud	Cane trash	Bagasse	C : N ratio	N per cent					
Group I	1A	3	1	...	34 : 1	1.08	Months 12.5	24.1	1.22	14.1
	1B	3	1	...	34 : 1	1.08	12.5	12.6*	1.08	12.5
	2A	6	1	1	36 : 1	1.07	8.2	18.6	1.19	9.1
	2B	6	1	1	36 : 1	1.07	9.0	20.0	1.21	9.3
	3A	3	...	1	38 : 1	1.05	7.5	21.3	1.21	9.6
	3B	3	...	1	38 : 1	1.05	7.5	13.0*	1.00	9.8
Group II	4A	3	1	...	34 : 1	1.08	6.2	30.3	1.27	18.4
	4B	3	1	...	34 : 1	1.08	6.2	30.6	1.20	17.3
	5A	6	1	1	36 : 1	1.07	7.1	24.6	1.17	17.3
	5B	6	1	1	36 : 1	1.07	7.1	27.2	1.22	16.9
	6A	3	...	1	38 : 1	1.05	7.3	25.7	1.17	17.4
	6B	3	...	1	38 : 1	1.05	7.3	26.2	1.16	18.6
Group III	7A	3	1	...	34 : 1	1.08	7.1	5.5*	1.01	11.9
	7B	3	1	...	34 : 1	1.08	7.1	12.9*	1.08	13.2
	8A	6	1	1	36 : 1	1.07	7.7	13.2*	1.05	14.6
	8B	6	1	1	36 : 1	1.07	7.7	5.7*	0.98	14.1
	9A	3	...	1	38 : 1	1.05	7.7	17.9	1.07	16.4
	9B	3	...	1	38 : 1	1.05	7.5	17.1	1.00	14.8
Group IV	10A	3	1	...	34 : 1	1.08	6.8	28.3	1.14	24.5
	10B	3	1	...	34 : 1	1.08	7.2	30.0	1.14	26.3
	11A	6	1	1	36 : 1	1.07	7.5	32.1	1.25	20.5
	11B	6	1	1	36 : 1	1.07	7.7	29.3	1.13	25.0
	12A	3	...	1	38 : 1	1.05	7.6	34.0	1.28	20.0
	12B	3	...	1	38 : 1	1.05	7.5	29.3	1.16	22.1

\*The heaps marked, got mixed with the mud paste, so that the per cent loss of dry matter is less than in other heaps, but this does not affect the results in the last column.

## RESULTS

From the results recorded in Table I, it can be readily seen that in all these experiments, loss of nitrogen and dry matter is much less than in those where composting is done only by aerobic fermentation. The average nitrogen content of the composts is also fairly satisfactory, being about 1.2 per cent. The period of composting is longer, but this is of no consequence since there is sufficient interval between the crushing season and the next planting. Complete stoppage of aeration after the initial rise of temperature is most beneficial for conserving nitrogen and dry matter, as in groups I and III. Placing the heaps in trenches is better than when composting is done on the ground.

Of these four methods, group I gives the best results and even this should not be very expensive. Any pit already available could be used in the factories and even otherwise, the expense of digging the pits would have to be incurred only once and there would be no recurring expenses, no turning being required at any time during the formation of the compost. These pits could be used over and over again during a number of years. The saving in loss of nitrogen and of dry matter would more than repay the initial cost of making these trenches.

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# \*STUDIES ON STORED GRAIN PESTS IN THE PUNJAB

## †II. BIOLOGY OF *BRUCHUS ANALIS* FAB. AND *BRUCHUS CHINENSIS* LINN. (*BRUCHIDAE*: *COLEOPTERA*)

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(With three text-figures)

### INTRODUCTION

**B**RUCHIDAE contains more than a 100 injurious species which occur in different parts of the world. Chittendon [1912], Garman [1917], Back [1930] and Bridwell and Bottimer [1933] have described seven species which are destructive to beans and peas in the United States of America. Bridwell [1918, 1920] has listed 11 injurious species from the Hawaii Islands; two of which were collected from imported seed. Skaif [1918, 1926] mentioned 12 species destructive to various leguminous seeds in South Africa. Wilson [1931] has found 12 species in Great Britain associated with seeds of garden plants. Zacher [1931, 1936] considers seven Bruchid species to be pests of stored products in Germany. Bonder [1936] has recorded 50 destructive species from Brazil. Bekman [1929] has mentioned 11 injurious species which he collected from imported seed in Russia. Ghosh [1937] has named four species to be harmful to various pulses in Burma. In India the following 11 injurious Bruchids have so far been recorded [Lefroy, 1909; Fletcher, 1916, 1917, 1923; Kunhi Kannan, 1912; Kasergod, 1919; Fletcher and Ghosh, 1919; Champion, 1919]: *Bruchus quadrimaculatus* Fab., *Bruchus affinis* Froel, *Bruchus phaseoli* Gyl., *Bruchus caeruleus* Champ., *Bruchus maculipyga* Champ., *Bruchus theobromae*, *Bruchus pisorum* Linn., *Bruchus emarginatus* All., *Bruchus analis* Fab., *Bruchus chinensis* Linn. and *Pachymerus gonagra* Fab. In the Punjab, we have so far collected only the three last-named species. Some of these Bruchids confer serious injuries

\*I. Observations on the reactions of the Dermestid beetle, *Trogoderma khapra* Arr., to light. *Indian J. Ent.* **1**, 57—63 (1939)

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on pulses and gram in storage. According to Fletcher and Ghosh [1919], generation after generation of *Bruchus chinensis* L. occur in the seed until there is hardly anything left of them. But in spite of their destructiveness and wide distribution practically nothing is known about them in India, and especially so in the Punjab. Because of their importance, studies on the biology of *Bruchus analis* F. and *Bruchus chinensis* L. were taken up and the results are presented in this paper.

### *Bruchus analis* F.

**Distribution.** *B. analis* F. has a limited distribution: so far it has been recorded from Germany, Rhodesia, Burma and India only. In India it has been collected from cowpeas and dried pulses in Mysore by Kunhi Kannan and Fletcher [1919, 1923] and from Ajnala, Banga, Gurdaspur, Gurgaon, Jhang, Jullundur, Karnal, Lahore, Lyallpur, Multan, Palampur, Panipat, Raiwind, Rohtak, Shergarh, and Sheikhpura in the Punjab by us.

**Food.** It feeds on a fairly wide variety of stored grains. Ghosh [1937] collected it from the following:—moong (*Phaseolus mungo*), lobia (*Phaseolus calcaratus*), mash (*Phaseolus radiatus*), moth (*Phaseolus aconitifolius*), peas (*Pisum sativum*), cowpeas (*Vigna catjang*), pigeon-peas [*Cajanus cajan* (*indicus*)], large white beans (*Dolichos lablab*), gram (*Cicer arietinum*), soy-bean (*Glycine hispida*) and sword bean (*Canavalia ensiformis*). In the Punjab it is a major pest of moong, mash, moth, peas and lobia.

### *Life-history*

**Copulation.** Copulation takes place immediately after emergence from the pupæ. Before the intimate connection is established the pair indulges in horse-play; the female runs away from the amorous male which, on persisting in its chase, receives a vigorous kick from its stouter spouse. Undaunted, the male resumes the chase again and after experiencing some more rebuffs ultimately succeeds in getting on to the back of the female and mating. During mating male stands in an upright position by supporting itself on its hinder pair of legs and the last abdominal segments which are modified for the purpose. After mating the female throws off the male to the ground where it lies on its back, the female in the meantime pulling out the aedagus gradually with its own hinder pair of legs. According to Ghosh [1937] copulation lasts for five to nine minutes; we, however, found it to last from 2·75 minutes to 17·5 minutes.

**Oviposition.** Females began to lay eggs singly or several of them together on the same grain any time within 72 hours of mating. Oviposition period varied from two to six, and four to twelve days during May to September and December respectively, depending upon temperature (Table I). A single female laid 11 to 150 eggs at the rate 1 to 82 eggs per day. The highest number of eggs were laid in August (an average of 95 eggs per female) and least in July and December (an average of 62·0 and 64·3 eggs per female respectively). Table I gives the oviposition period and the total number of eggs laid by a female in her life-time and daily for each month from March to December.

TABLE I  
*Oviposition record of Bruchus analis Fab.*

Month	Number of Observations	Total number of eggs laid			Number of eggs laid daily			Oviposition period in days		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
March to April	14	44	119	87.6	4	40	14.4	3	9	6.0
May	21	56	118	89.0	1	56	24.8	2	6	3.75
June	14	50	103	83.9	1	82	23.3	3	5	3.40
July	15	11	90	62.0	2	72	18.0	2	5	3.40
August	17	55	150	95.0	2	68	23.7	3	6	4.0
September	13	63	105	75.0	2	65	21.0	3	5	4.1
October	10	39	113	91.2	1	50	15.7	3	8	5.8
November	10	64	115	88.3	3	41	16.0	6	8	6.8
December	10	50	85	64.3	1	31	8.5	1	12	7.5

During May to September a female died immediately, but during April and October to December she lived for two to five days, after laying its last egg.

*Hatching.* Duration of the egg-stage varied with the season : eggs laid in May to August hatched in three to six days, those laid in April, September and October in four to eight days while those laid in November and December took 8 to 13 and 18 days respectively to hatch (Table II). On hatching, the larva bored directly into the grain by cutting out a hole in that side of the egg-shell which was in contact with the grain. Frass produced by the larva in making its bore accumulated in the empty egg-shell which remained sticking to the grain after hatching.

TABLE II  
*Duration of the egg-stage of Bruchus analis Fab.*

Month	Number of observations	Duration of egg-stage (in days)			Remarks
		Minimum	Maximum	Average	
March to April	1227	4	8	6.3	Only two observations ; eggs hatched simultaneously
May	1869	3	5	4.0	
June	1188	3	4	3.7	
July	928	3	5	3.7	
August	1617	3	6	4.2	
September	1370	4	7	5.3	
October	912	6	8	6.5	
November	806	6	13	6.5	
December	386	..	..	18.0	

*Viability of eggs.* Viability of eggs is presented graphically in Fig. 1. It was at its highest in May and lowest in August and during the active period (April to October) it was found to be inversely proportional to relative humidity.

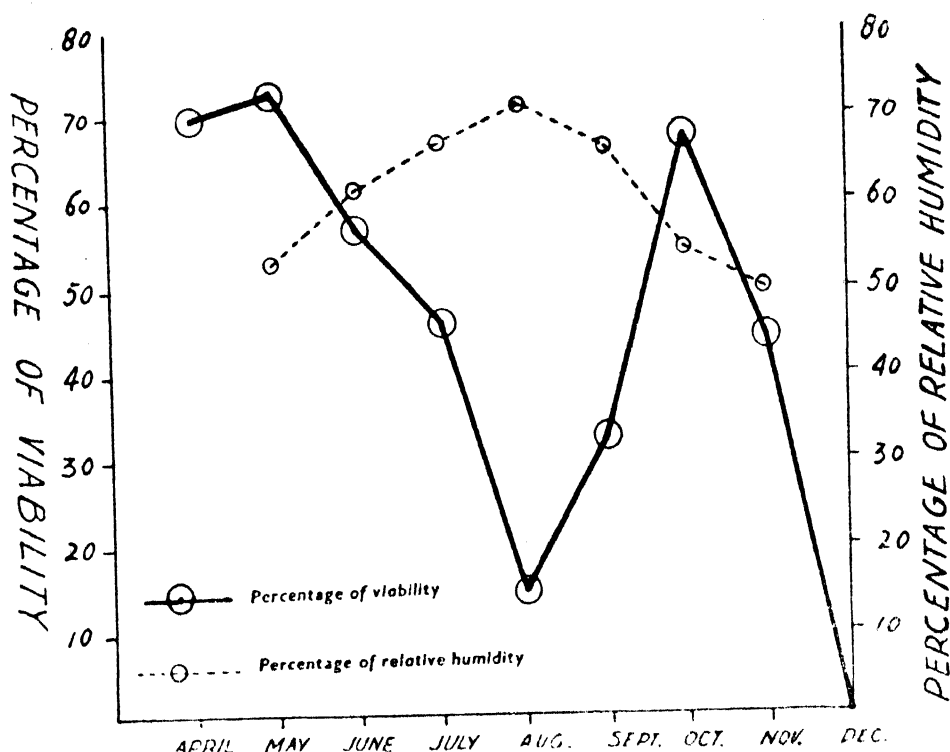


FIG. 1. Viability of eggs of *Bruchus analis* F. in different months

*Larval stage.* The entire larval stage was passed inside a grain. When full-fed, the larva migrated towards the periphery and came to lie next to the seed coat where it pupated. Duration of the larval stage during different months is given in Table III.

TABLE III

*Duration of the larval stage of B. analis Fab.*

Month	Number of observations	Larval stage in days		
		Minimum	Maximum	Average
April	343	12	25	17.4
May	824	9	31	13.4
June	605	9	24	13.4
July	391	9	26	13.7
August	209	8	21	12.5
September	432	9	30	15.7
October	321	11	27	17.9
November	367	16	31	23.6
December	108	26	43	35.4

It will be observed from Table III that the duration of the larval stage is the shortest during June to September and longest in December. The pest passed the winter as hibernating larvæ and it was observed that all those larvæ which hatched on and after the middle of November entered into hibernation. A few typical cases of the duration of the over-wintered larvæ are presented in Table IV.

TABLE IV  
*Life of the over-wintered larvæ*

Date of hatching	Date of pupation	Life of the over-wintered larvæ (in days)
21 October 1940	22 March 1941	152
28 October 1940	22 March 1941	145
11 November 1940	23 March 1941	112
14 November 1940	22 March 1941	129
21 November 1940	15 March 1941	103
3 December 1940	7 March 1941	96

*Pupal stage.* Pupal stage, like the larval stage, was also passed inside the grain. Duration of the pupal stage in different months of the year is given in Table V.

TABLE V  
*Duration of the pupal stage of B. analis F.*

Month	No. of observations	Duration of pupal stage (in days)		
		Minimum	Maximum	Average
March	114	8	23	15.6
April	293	5	14	7.3
May	523	6	14	8.3
June	392	6	13	8.2
July	281	5	14	8.4
August	165	5	11	7.5
September	186	6	13	7.4
October	172	7	15	10.5
November	196	12	23	18.9
December	119	14	36	22.3

It will be observed from the above table that the pupal stage is completed, on an average, in about 7.5 days during August to September and in 22.3 days during December.

*Longevity of adults.* The female adults, on an average, lived for 4.8 days during May to July and 16.1 days in December. The males lived longer than the females, i.e. for 6 to 20.4 days.

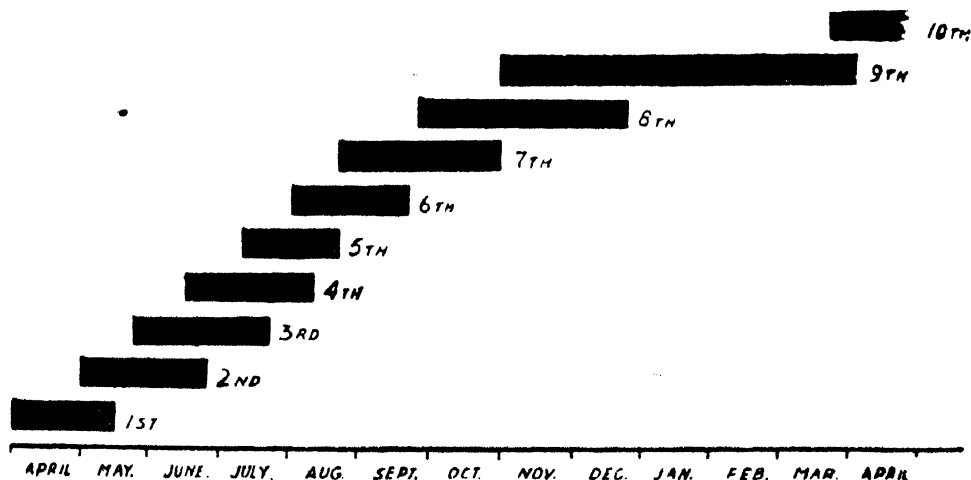
TABLE VI

*Longevity of males and females in different months*

Month	Number of Observations	Longevity of female adults (in days)			Longevity of male adults (in days)		
		Minimum	Maximum	Average	Minimum	Maximum	Average
April	14	5	11	8.0	6	13	9.4
May	19	3	7	4.8	5	8	6.2
June	17	3	7	4.8	3	9	6.0
July	15	3	7	4.8	5	11	6.8
August	10	3	7	5.3	5	10	7.7
September	13	4	7	5.7	7	10	8.3
October	10	5	11	7.4	9	14	11.0
November	10	7	12	9.5	12	12	13.0
December	10	6	22	16.1	11	30	20.4

*Seasonal history and number of generations.* The females appeared in March when they laid eggs; the earliest date on which the adults emerged was March 6. The annual calendar of activities of the pest is given below :

March . . . . .	Activity of the pest begins
April to August . . . . .	All stages of the pest present. Damage by it at its maximum
September . . . . .	All stages present but its activity shows distinct decline
October . . . . .	All stages present, activity greatly reduced, some of the larvæ which hatch out in the last week enter hibernation
November . . . . .	All stages present, activity very much reduced, all larvæ hatching on November 15 and after enter hibernation
December . . . . .	All stages present, activity at lowest ebb, eggs do not hatch
January to February . . . . .	Hibernating larvæ only present, activity nil

FIG. 2. Number of generations of *B. analis* F.

During March to December the pest passed through nine to ten overlapping generations (Fig. 2).

*Sex ratio in different generations.* Sex ratio varied in different generations and at different times of the year. In the first, second, third, fourth, ninth and tenth generations (i.e. during April to July, November to March) females predominated and in the fifth and sixth generations (i.e. from July to September) males predominated but in the seventh and eighth generations (September to October) the female and male population was at par.

The sex ratio of *B. analis* Fab. in different generations is given in Table VII.

TABLE VII  
*Sex ratio of B. analis* Fab. in different generations

No. of generations	Total number of insects counted	Number of males	Number of females	Sex ratio	
				Male	Female
I	363	150	213	41.3	58.7
II	814	321	493	39.4	60.6
III	353	136	217	38.6	61.5
IV	333	159	174	47.7	52.3
V	186	107	79	57.5	42.5
VI	74	44	30	59.4	40.6
VII	280	140	140	50.0	50.0
VIII	552	275	277	49.8	50.2
IX	137	58	79	42.3	57.7
X	237	103	134	43.4	56.6

*Development in various leguminous seeds.* Development of the pest was studied in the following 11 leguminous seeds:—moong (*Phaseolus mungo*), moth (*Phaseolus aconitifolius*), gram [both *desi* and *kabuli* (*Cicer arietinum*)], peas (*Pisum sativum*), lobia (*Phaseolus calcaratus*), mash (*Phaseolus radiatus*), arhar [*Cajanus cajan* (*indicus*)], lentil (*Lens esculenta*), sem (*Dolichos lablab*) and guara (*Cyamopsis psoralioides*). It failed to breed on *sem* and *guara*. On lentil, out of 100 eggs laid and hatched, only one larva was able to attain the adult stage. The shortest duration of the larval and pupal stages was observed on *lobia*, *arhar*, *moth*, *kabuli* gram and *moong*; and longest on *mash*. *Lobia* appeared to be its most favoured food. Ghosh [1937]

observed that cowpeas were liked most by this insect since the duration of its life-cycle was shorter on it as compared to that on *Cajanus cajan* (*indicus*), *Dolichos* and *Phaseolus mungo*.

TABLE VIII

*Comparative rate of development and percentages of larvæ and pupæ completing their growth successfully on different seeds*

Food	Duration of combined larval and pupal stages (in days)			Percentages of larvæ and pupæ completing the stage successfully
	Minimum	Maximum	Average	
<i>Moong</i>	17	45	27.5	72.1
<i>Mash</i>	19	59	41.5	76.1
<i>Moth</i>	17	34	25.7	70.1
<i>Kabuli gram</i>	18	45	25.8	73.3
<i>Desi gram</i>	30	44	36.9	10.9
<i>Peas</i>	20	44	32.1	54.6
<i>Lobia</i>	13	45	22.8	87.8
<i>Arhar</i>	17	33	24.1	65.7

*Nature and extent of damage.* On hatching, the larvæ bored into the seed and by the time they completed their development, they consumed the entire contents of the grain, leaving only the outer shell behind: the adults escaped by cutting out a circular hole in this shell. As generation after generation was passed in quick succession during the active season, and as only one larva was found in a single grain, the entire quantity of stored grains was found to be reduced to a mass of hollowed-out seeds each with a circular hole at one end. When grain was stored in air-tight receptacles, a foul smelling fungus also developed on the seeds.

#### *BRUCHUS CHINENSIS* Linn

*Distribution.* *B. chinensis* L. is of world-wide distribution. It is reported from the United States of America, Mauritius, Hawaii, England, Germany, Porto Rico, Rhodesia, Santo-Domingo, Formosa, Africa, China, Philippine, Japan, Java, Ceylon, Burma and India. In the Punjab, we have collected it from Panipat, Multan, Karnal, Gurgaon, Jhang, Palampur, Banga and Lyallpur.

*Food.* In the Punjab it has been found damaging gram (*Cicer arietinum*), moong (*Phaseolus mungo*), moth (*Phaseolus aconitifolius*), mash (*Phaseolus radiatus*), lobia (*Phaseolus calcaratus*), peas (*Pisum sativum*), cowpeas (*Vigna catjang*), lentil (*Lens esculenta*) and arhar [*Cajanus cajan* (*indicus*)]. Elsewhere it has also been recorded doing damage to chicken pea, *sem* (*Dolichos lablab*), *Dolichos biflorus*, soy bean (*Glycine hispida*), chickling vetch (*Lathyrus sativus*), *Vicia faba*, *Arachis hypogaea*, cotton bolls, cotton seeds, sorghum, millet and maize.

*Life-history*

*Copulation.* Copulation takes place immediately after emergence, the pairs remaining in coitus for 4·8 to 16 minutes.

*Oviposition.* Females started laying eggs next day after copulation, their mode of oviposition being identical with that of *B. analis* F. Usually more than one egg were laid on a single grain of gram and, unlike *B. analis*, two or three larvæ were found to develop in their individual chambers in the same grain. A single female laid 34 to 113 eggs at the rate of 1 to 37 eggs per day. The highest number of eggs were laid in May and October and least in April, June, July and December. Table IX gives oviposition record of this pest.

TABLE IX  
*Oviposition record of B. chinensis L.*

Month	Number of Observations	Total number of eggs laid			No. of eggs laid daily		
		Minimum	Maximum	Average	Minimum	Maximum	Average
April	16	34	85	55·2	1	9	6·0
May	17	66	111	90·4	1	87	10·0
June	18	39	94	62·7	2	33	12·0
July	15	35	83	67·0	1	31	11·3
August	16	39	99	74·3	1	23	13·2
September	15	46	104	87·7	1	24	11·7
October	15	56	113	92·6	1	24	9·4
November	8	54	95	69·2	1	13	4·9

*Hatching.* Egg stage occupied seven to fourteen days in April, four to six days in September, and eight to sixteen days in November.

TABLE X  
*Duration of the egg-stage of B. chinensis L.*

Month	No. of observations	Duration of egg-stage (in days)		
		Minimum	Maximum	Average
April	596	7	14	9·5
May	48	5	9	6·5
June	62	6	9	7·2
July	43	6	9	6·8
August	225	4	7	5·7
September	729	4	6	4·8
October	593	5	9	7·0
November	151	8	16	13·1

*Larval stage.* Larval stage was passed inside the grain. When full-grown, the larva migrated towards the periphery of the grain and rested just beneath the seed coat. Duration of the larval stage was the shortest in August and September and the longest in November (Table XI).

TABLE XI

*Duration of the larval stage of B. chinensis L.*

Month	No. of observations	Duration of the larval stage (in days)		
		Minimum	Maximum	Average
April	30	17	21	18.4
May	40	12	33	20.0
June	19	14	20	17.0
July	71	14	26	16.5
August	266	10	21	12.0
September	586	10	21	13.5
October	313	14	30	20.0
November	21	26	38	34.9

The pest hibernated as larva from October onwards. Hibernation usually began on October 10 and reached its climax on 15 when all the larvæ which hatched on this date and after hibernated. In all 423 larvæ were kept under observation and 62.4 per cent of this lot over-wintered and emerged as adults successfully. A few typical cases of the duration of the over-wintered larvæ are given in Table XII.

TABLE XII

*Life of the over-wintered larvæ.*

Eggs hatched on	Larvæ pupated on	Life (in days) of the over-wintered larvæ
14 October 1940	15 March 1941	152
14 October 1940	27 March 1941	164
17 October 1940	15 March 1941	149
20 October 1940	6 April 1941	168
21 October 1940	15 March 1941	145
4 November 1940	15 March 1941	131
4 November 1940	3 April 1941	150
13 November 1940	27 March 1941	134
21 November 1940	23 March 1941	122
28 November 1940	27 March 1941	120
2 December 1940	29 March 1941	117
2 December 1940	3 April 1941	122

*Pupal stage.* Pupal stage was also passed inside the grain, the adults escaping from the grain by cutting out a circular hole in the seed coat. Duration of the pupal stage in different months is given in Table XIII,

TABLE XIII  
*Duration of the pupal stage of B. chinensis L.*

Month	No. of observations	Duration of pupal stage (in days)		
		Minimum	Maximum	Average
May	375	6	13	8.1
June	31	6	9	7.9
July	56	5	8	6.4
August	162	4	9	6.4
September	543	6	15	8.9
October	348	8	18	11.9
November	101	15	28	18.2

*Longevity of adults.* Females lived longer than the males. Longevity of the adults in different months of the year is given in Table XIV.

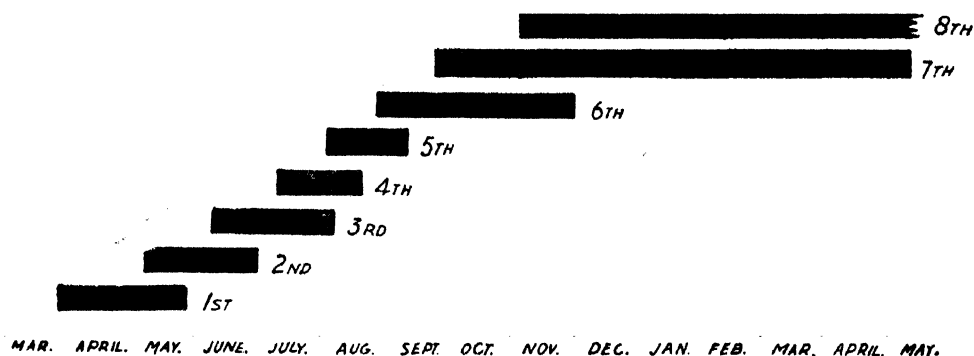
TABLE XIV  
*Longevity (in days) of adults in different months*

Month	No. of observations	Male			Female		
		Minimum	Maximum	Average	Minimum	Maximum	Average
April	16	6	17	10.7	6	18	13.0
May	17	6	9	7.0	5	8	6.4
June	18	3	9	6.1	4	8	6.2
July	15	5	7	6.1	5	8	7.1
August	16	5	7	5.9	5	8	6.5
September	15	5	12	7.4	5	12	8.4
October	15	6	12	9.7	9	12	10.6
November	8	12	20	16.2	13	20	15.5

*Seasonal history and number of generations.* The adults began to appear towards the end of March, the earliest date of their appearance being 26. Seasonal history is given below :

- March . . . . . Adults emerge towards the end
- April to September . . . . . All stages of the pest are present. Damage by it is at its maximum. In May to July viability of the eggs falls considerably
- October to November . . . . . All stages present, activity reduced. Some larvae begin to hibernate
- December to February . . . . . Hibernating larvæ only present

The pest passed through seven to eight generations in a year which overlapped (Fig. 3)

FIG. 3. Number of generations of *B. chinensis* L.

*Sex ratio in different generations.* The males, as is seen from Table XV, predominate in all the generations.

TABLE XV  
*Sex ratio of B. chinensis L. in different generations*

No. of generation	Total number of insects counted	Number of males	Number of females	Sex ratio	
				Male	Female
I	864	453	411	52.4	47.6
II	2756	1508	1248	54.7	45.3
III	2592	1423	1169	54.9	45.1
IV	2437	1449	988	59.5	40.5
V	4692	2622	2070	55.9	44.1
VI	7295	3765	3530	51.6	48.4
VII	3204	1683	1521	52.5	47.5

*Development in various leguminous seeds.* Development of the pest was studied in the following 11 leguminous seeds: moong (*Phaseolous mungo*), moth (*Phaseolus aconitifolius*), gram both *desi* and *kabuli* (*Cicer arietinum*), peas (*Pisum sativum*), lobia (*Phaseolus calcaratus*), mash (*Phaseolus radiatus*), arhar [*Cajanus cajan* (*indicus*)], lentil (*Lens esculenta*), sem (*Dolichos lablab*) and guara (*Cyamopsis psoralioides*). It failed to breed in sem and guara. It developed most quickly in moth, lobia, moong, and very slowly in mash and peas. It laid the least number of eggs on lentil. Ghosh [1937] observed the shortest duration of this insect in pigeon-peas [*Cajanus cajan* (*indicus*)] which was also found by him to suffer most from its ravages. Table XVI gives

the comparative rate of development and oviposition of this pest along with the percentages of larvæ and pupæ completing their growth successfully in different seeds.

TABLE XVI

*Comparative rate of development, oviposition and percentages of larvæ and pupæ completing their growth successfully in different seeds*

Variety of grain	Duration of larval and pupal stages (in days)			No. of eggs laid			Viability of the larval and pupal stage
	Minimum	Maximum	Average	Minimum	Maximum	Average	
Kabuli gram	22	31	26.5	58	89	78.0	92.4
Desi gram	20	29	24.0	52	97	77.4	91.9
Peas	25	66	43.3	53	101	79.0	42.3
Arhar	21	26	23.3	66	106	81.8	95.8
Moong	20	27	22.1	58	105	77.8	97.0
Moth	21	24	21.6	66	82	73.0	96.0
Lentil	22	36	26.6	51	70	61.3	65.0
Mash	36	49	43.0	32	100	63.4	87.6
Lobia	21	25	21.6	60	90	77.0	93.3

*Nature and extent of damage.* Nature and extent of damage was found to be identical with that of *Bruchus analis* F. In a single grain there were found as many as eight larvæ.

#### NATURAL ENEMIES

Both the insects were found to be parasitized by *Bruchobius laticeps* Ashm. (family, Miscogasteridæ) Order Hymenoptera in their larval stages. We collected this parasite from Panipat, Karnal, Shergarh, Lahore, Lyallpur and Jhang.

#### SUMMARY

*Bruchus analis* F. and *Bruchus chinensis* L. are the two important pests of various stores, pulses and gram in the Punjab. The former remains active from March to November whereas the latter is active from March to October. The eggs are glued to the grain and the larva on hatching bores into the seed and feeds on its contents. When full grown, it migrates towards the periphery and comes to lie next to the seed coat where it pupates. The adult emerges by cutting out a circular hole in the seed coat.

In the case of *B. analis* F., a single female lays 11 to 150 eggs in 2 to 12 days at the rate of 1 to 82 eggs per day. Incubation period lasts from 1 to 18 days, larval stage occupies 8 to 43 days and pupal stage is completed in 5 to 36 days depending upon season. Males live longer than the females. There are 9 to 10 generations in a year and these overlap.

A single female of *B. chinensis* L. lays 34 to 113 eggs at the rate of 1 to 37 eggs per day. Eggs hatch in 4 to 16 days, larvæ are full grown in

10 to 38 days, whereas pupal stage occupies 4 to 28 days. Longevity of the female adult varies from 4 to 20 days and that of male from 3 to 20 days. The insect passes through seven to eight overlapping generations in a year.

Development of these insects on 11 different leguminous seeds is also described. The larvæ of both these insects are parasitized by *Bruchobius laticeps* Ashm.

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# STUDIES ON THE QUALITY OF JAYWANT COTTON GROWN FROM SEEDS OBTAINED FROM DIFFERENT STAGES OF PROPAGATION

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IN order to maintain the purity of improved varieties of cotton, a subsidized scheme 'Maintenance of the nucleus of pure seed' is established by the Indian Central Cotton Committee, Bombay. For this purpose, the practice followed in the Southern Maratha Country is to sow in two-acre plot, on the Dharwar Farm, seed of a particular variety inbred by the Cotton Breeder. The plants in this stage known as stage I are selfed, the inbred bolls are picked separately and ginned and the seed is then multiplied on the same farm as well as in Dharwar taluka on an area of about 25 acres at each of these places. The produce of this seed is known as stage II and is handed over to the Cotton Superintendent from the farm for multiplication in all the cotton centres by registered growers under expert supervision. The plants of the succeeding crops are harvested and ginned under supervision so as to eliminate all chances of admixture with foreign seed. This ensures a fairly high degree of purity in the seed which ultimately reaches the farmers' hands and as the process is continuous, the deterioration by mixture can hardly take place.

It may, however, be noted that during the first and second stages, there is rigid control over the purity of the seeds, but due to the numerous uncontrollable factors in the districts, the cotton may not remain quite pure in subsequent stages. The roguing of foreign cotton plants is done up to the fourth stage and the number of plants removed per acre are on an average of about 0.5 and 1.3 per cent in the third and fourth stages, respectively.

It may be mentioned that the produce up to the fifth stage only is under the control of the Agricultural Department and the area under Jaywant cotton in that stage is 1,18,000 acres. The produce of this area is considered sufficient for sowing about 12,00,000 acres which is the total area under Kumpta cotton. Thus, the sixth stage from which deterioration takes place is left out.

It should not be forgotten that there are chances of admixture from the small area of different varieties of cotton grown in Bijapur, Dharwar and Belgaum districts and due to importation of cotton from the Nizam's Dominions as well.

Thus, the chances of mixture are :—(1) Natural cross pollination, (2) Mechanical mixture when handled from the field to the seed sowing stage such as heaping the *kapas* (seed cotton) in factories, ginning in factories and storage of seed.

### OBJECT OF THE INVESTIGATION

Jaywant cotton is grown on about 12,00,000 acres in the Bombay-Karnatak. The extensive cultivation of this variety has been possible on account of its superiority in the economic characters over other local varieties. There was, however, an impression (not based on scientific evidence) that the crop from later stages deteriorated in quality. The investigation was, therefore, undertaken to study the variations of fibre and agronomic characters of the cotton grown from seeds obtained from different stages of multiplication and the results are presented in this paper.

### MATERIAL AND METHOD

The experiment was conducted on the Dharwar Farm on an area of about half an acre. Inbred seed was obtained from the Cotton Breeder, second stage from the Superintendent, Dharwar Farm, and the seed of later stages, i.e. from third to sixth was supplied by the Cotton Superintendent from the Hubli centre, which is the biggest centre in the whole tract, and the results are, therefore, applicable to the whole of the Kumpta tract. The seed samples of each stage were sufficient for sowing about three *gunthas* and are considered representative of each stage. The experiment was commenced in 1938-39 season and was continued in the next year. During these two seasons, the seed of all the six stages was sown in duplicated plots yielding 12 samples for each season. Two representative samples, each weighing about two pounds of *kapas* of each stage from the second picking were obtained.

The problem was studied from the following points of view and the methods and procedure followed were the same as adopted at the Technological Laboratory, Mutanga, for measuring the different fibre characters. The maturity ratio and standard hair-weight were calculated by using the formula derived by Pierce and Lord [1934]. The number of fibres were also calculated from the values of fibre length, fibre-weight and weight of lint per seed.

#### *Fibre characters :*

- (1) Mean fibre length in inches
- (2) Fibre-weight per unit length
- (3) Fibre maturity
- (4) Maturity ratio
- (5) Standard hair-weight

#### *Agronomic characters :*

- (1) Ginning percentage
- (2) Lint index
- (3) Number of fibres per seed

#### *Experimental results :*

The results of fibre and agronomic characters of Jaywant cotton grown from seeds obtained from different stages of propagation are given in Tables I and II.

TABLE I  
*Fibre characters*

Season	Stage											
	I		II		III		IV		V		V	
	1	2	1	2	1	2	1	2	1	2	1	2
<i>1. Mean fibre length (inches)</i>												
1938-39	0.91	0.90	0.90	0.89	0.91	0.91	0.91	0.92	0.91	0.91	0.91	0.91
1939-40	0.91	0.94	0.93	0.91	0.92	0.91	0.91	0.91	0.91	0.92	0.90	0.91
<i>2. Mean fibre-weight per unit length</i>												
1938-39	0.171	0.177	0.178	0.163	0.173	0.170	0.179	0.174	0.183	0.178	0.188	0.190
1939-40	0.182	0.182	0.189	0.190	0.190	0.187	0.193	0.198	0.191	0.183	0.207	0.202
<i>3. Fibre maturity (per cent mature hairs)</i>												
1938-39	68	67	67	62	64	66	64	63	67	69	67	71
1939-40	67	65	68	69	65	65	64	67	66	67	69	67
<i>3. Fibre maturity (per cent immature hairs)</i>												
1938-39	23	24	22	26	25	24	24	26	24	21	21	21
1939-40	23	24	22	21	25	23	25	23	24	22	22	22
<i>4. Maturity ratio</i>												
1938-39	0.957	0.951	0.958	0.930	0.939	0.948	0.943	0.933	0.951	0.967	0.961	0.972
1939-40	0.954	0.945	0.961	0.967	0.942	0.949	0.939	0.954	0.948	0.958	0.963	0.956
<i>5. Standard hair-weight</i>												
1938-39	0.178	0.186	0.185	0.175	0.186	0.179	0.190	0.186	0.192	0.184	0.196	0.195
1939-40	0.191	0.192	0.196	0.196	0.202	0.197	0.205	0.207	0.202	0.191	0.215	0.211

TABLE II  
*Agronomic characters*

Season	Stage											
	I		II		III		IV		V		VI	
	1	2	1	2	1	2	1	2	1	2	1	2
<i>1. Ginning percentage</i>												
1938-39	29.9	30.5	30.5	30.2	29.0	29.0	29.5	29.7	29.5	29.2	28.6	29.0
1939-40	29.5	30.4	29.0	30.7	28.6	29.4	27.5	27.0	28.2	29.8	27.0	27.5
<i>2. Lint index</i>												
1938-39	2.70	2.80	2.74	2.69	2.56	2.57	2.50	2.67	2.45	2.18	2.36	2.52
1939-40	2.57	2.48	2.25	2.34	2.30	2.60	2.23	2.16	2.35	2.42	1.97	2.13
<i>3. Number of fibres per seed</i>												
1938-39	6170	6215	6050	6580	6390	5870	5400	5890	5380	4610	4910	5110
1939-40	5480	5160	4500	4760	4670	5370	4460	4240	4780	5080	3740	4100

## DISCUSSION OF RESULTS

The analysis of variance was applied to the results obtained for the various fibre and agronomic characters of Jaywant cotton studied in this paper and the significance of the differences have been judged according to this method. As the significant effect between different stages does not necessarily indicate deterioration, the separation of linear component due to deterioration was also worked out to study the effect due to different stages of propagation.

TABLE III

*Summary of results*

Fibre and agronomic characters	Stages						Average	Standard error
	I	II	III	IV	V	VI		
Fibre length . . . . .	0.915	0.908	0.912	0.912	0.912	0.908	0.911	0.0040
Fibre-weight . . . . .	0.178*	0.180	0.180	0.186	0.184	0.197*	0.184	0.0022
Percentage of mature hairs . . . . .	67	66	65	65	67	68*	66.2	0.85
Maturity ratio . . . . .	0.952	0.954	0.945	0.942	0.956	0.963	0.952	0.007
Standard hair-weight . . . . .	0.187*	0.188*	0.191	0.197	0.192	0.204*	0.193	0.0022
Ginning percentage . . . . .	30.1*	30.1*	29.0	28.4	29.2	28.0*	29.1	0.292
Lint index . . . . .	2.64*	2.51	2.51	2.39	2.35	2.25*	2.44	0.054
No. of fibres per seed . . . . .	5756*	5472	5575*	4998	4962	4465*	5205	157

\* Indicate significantly higher or lower values than the mean

The analysis of variance for fibre length, fibre-weight, percentage of mature hairs, maturity ratio and standard hair-weight are given in Table IV.

From Table IV, it is evident that the variance due to stages is non-significant for fibre length, percentage mature hairs and maturity ratio and that it is significant for fibre-weight per unit length and standard hair-weight. The standard hair-weight is calculated from fibre-weight; the significant values for standard hair-weight can, therefore, be expected.

The analysis of variance results of fibre-weight and standard hair-weight were further examined and the analysis is given in Table V.

The linear component being significant for fibre-weight and standard hair-weight shows evidence of deterioration. But reference to Table I will show that the values in the sixth stage are rather high during both the seasons. It may be noted that higher fibre-weight means coarser cottons which in turn will give lower spinning value if all other fibre properties remain the same. Hence it can be seen that the cotton from the sixth stage is particularly inferior as judged from the fibre-weight and standard hair-weight values. This is corroborated from the summary of results given in Table III where it can be observed that only the sixth stage values are significantly higher than the average.

TABLE IV  
*Analysis of variance*  
(Fibre characters)

Sources of variation	Degrees of freedom	Fibre length		Fibre-weight		Percentage mature hairs		Maturity ratio		Standard hair-weight	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square
Due to seasons	1	0.0004 <sup>**</sup>	0.00040	0.001204	0.001204 <sup>**</sup>	0.66	0.66	0.000028	0.000028	0.001247	0.001247 <sup>**</sup>
Due to stages	5	0.0002	0.00004	0.000936	0.000188 <sup>**</sup>	43.33	8.67	0.001170	0.000234	0.000844	0.000169 <sup>**</sup>
Seasons × stages	5	0.0006	0.00016	0.000154	0.000031	24.84	4.97	0.000563	0.000113	0.000090	0.000018
Field error	12	0.0009	0.00008	0.000224	0.000019	35.00	2.92	0.000059	0.0000080	0.000231	0.000019

<sup>\*\*</sup> Denotes significance for 1 per cent level

TABLE V  
*Further analysis of variance*

	Degrees of freedom	Fibre-weight		Standard hair-weight	
		Sum of squares	Mean square	Sum of squares	Mean squares
Linear regression . . . . .	1	0.000704	0.000704*	0.000641	0.000641*
Deviation from regression . . . . .	4	0.000234	0.000058	0.000203	0.000051
Total (stages) . . . . .	5	0.000938		0.000844	

\* Denotes significance for 1 per cent level

It is, therefore, apparent that there is no evidence for deterioration of Jaywant cotton in the first five stages as judged by the various fibre characters studied in this paper. The only variation is due to seasons with which the present study is not concerned.

It may be observed that Jaywant cotton did not show any decline, in fibre length, percentage of mature hairs and maturity ratio for different stages of propagation.

The fibre-weight values of 1938-39 season showed a tendency for the cotton to become coarser and it is interesting to note that it is coarsest in the sixth stage for both the seasons. It may be noted that 1938-39 samples were finer than those of the other season and this may possibly be due to climatic factors.

The standard hair-weight, which is a derived property, showed a slight increasing tendency in the successive stages for both the seasons, the sixth stage recording the highest values in both seasons. Like fibre-weight, these values are higher in 1939-40.

#### *Agronomic characters*

It can be seen from Table II that the result of the ginning percentage, lint index and number of fibres per seed showed a decreasing tendency from successive stages, the sixth stage giving the lowest values for both the seasons. The number of fibres per seed is derived from fibre-weight and lint-index values and as these are correlated to each other, the lower number of fibres in the sixth stage can be ascribed to the higher fibre-weight values obtained for that stage. Needless to mention that the lower ginning percentage, lint index and number of fibres per seed go to show deterioration.

The analysis of variance due to stages show significant variation. This was further examined and the analysis of variance is given in Table VII.

It can be seen from Table VII that the linear component is also significant for the agronomic characters studied indicating deterioration.

But like fibre characters, the results in Table III indicate that only in the sixth stage, the ginning percentage is significantly lower than for other stages. This is corroborated by the results of the lint index and number of

fibres per seed which are significantly lower in the sixth stage only. It, therefore, seems evident that the agronomic characters also did not show any decline in the first five stages of propagation.

TABLE VI  
*Analysis of variance of agronomic characters*

Sources of variation	Degrees of freedom	Ginning percentage		Lint index		Number of fibres per seed	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square
Due to seasons	1	4.166	4.166†	0.3602	0.3602†	6237301	6237301†
Due to stages	5	14.283	2.86†	0.3857	0.0771†	4647180	929436†
Seasons × stages	5	4.194	0.84	0.1891	0.0378	1464631	293006
Field error	12	4.070	0.34	0.1408	0.0117	1177162	98096

† Denotes significance for 1 per cent level

TABLE VII  
*Further analysis of variance*

Sources of variation	Degrees of freedom	Ginning percentage		Lint index		Number of fibres per seed	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of squares	Mean square
Linear regression	1	10.656	10.656†	0.3730	0.3730†	4194893	4194893
Deviation from regression	4	3.627	0.907	0.0127	0.0032	452287	113072

† Denotes significance for 1 per cent level

### CONCLUSIONS

Jaywant cotton grown on the Dharwar Farm from seeds obtained from six stages of propagation were examined for fibre and agronomic characters during 1938-39 and 1939-40 seasons. It is found that there is no evidence of deterioration in fibre length, fibre maturity and maturity ratio but there is a tendency for the cotton to become coarser, to give lower ginning percentage lower lint index and lesser number of hairs per seed during the later stages of propagation.

It is clear that there is no deterioration in the first two stages which are definitely pure and for the later stages where rigid control may not be possible, there is a slight tendency for the cotton to deteriorate. It may be noted that in respect of these characters, the values of the sixth stage are significantly

inferior to those of the other stages, indicating deterioration in the sixth stage only. It may be stated that the Department is using the seeds up to the fifth stage only whereafter visible deterioration takes place in the economic characters studied. The system of auction sale adopted in this tract is after grading and generally it is observed that the cotton up to the third stage does not go into the lower grade which corroborates with the findings in this study. The values of the maturity ratio, standard hair-weight and number of fibres per seed are derived by calculation from fibre-weight and other measureable characters.

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# EFFECT OF LIMING ON THE TRANSFORMATION OF PHOSPHORUS IN ACID SOILS

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**T**HAT application of lime influences the quantity of available phosphorus in soils has been pointed out by several workers, both by laboratory analysis and field experiments. Naftel [1937] showed that readily available phosphorus by Truog's method was greatly increased (more than doubled) by the addition of lime. Askinazi and Yarusov [1928] showed that introduction of lime in a podsolized soil resulted in an accumulation of the mineral phosphoric acid in the soil solution and an increased phosphate solubility in weak acids. Cook [1935] showed that the addition of lime to soils caused significant increases in the amounts of readily available soil phosphates.

Sewell and Latshaw [1931] found that fertilization with superphosphates did not increase the percentage of phosphorus in alfalfa but that application of lime with the superphosphate did. Albrecht and Klemme [1939] reported field work conducted with lespedeza forage in which application of limestone and superphosphate almost doubled the calcium, phosphorus and nitrogen content of the crop over that obtained from superphosphate alone. Davis and Brewer [1940] found that liming soils low in calcium content enabled winter legumes to utilize larger quantities of the phosphorus supplied by superphosphate.

Very little is, however, known as to the precise nature of the transformation process and also as to the type of phosphorus compounds that contribute towards the increased availability. It has been suggested by some that a part of the iron and aluminium phosphates becomes soluble by chemical interaction with lime, while according to others it is due to the mineralization of organic phosphorus compounds present in the soil. In a study of the distribution of different forms of phosphorus in some Indian soils it has been found by the authors that acid soils are characterized by high accumulation of organic phosphorus and also by a high percentage of iron and aluminium phosphates. Transformation of one or both of these types of compounds seems to be the most probable thing to happen during the process of conversion. The change in the soil reaction brought about by lime is of course a fundamental factor in both these kinds of transformation.

The object of this work was to find out the nature of transformation effected by liming, by determining the relative amounts of the different groups of phosphorus compounds by fractionating the soil with and without liming treatments. The soil selected was a paddy soil from Titabari, Assam. The pH of the soil is 4.7 and its content of  $P_2O_5$  is 0.1145 per cent. The available phosphorus is extremely low, it being 2.6 mg.  $P_2O_5$  per 100 gm. of soil.

The liming materials used were chemically pure calcium carbonate, calcium hydroxide, calcium sulphate and magnesium oxide each at the rate of 2.5, 5 and 7.5 tons per acre.

## PROCEDURE

The general procedure adopted was as follows: 20 gm. samples of soil were weighed into wide-mouthed flasks and thoroughly mixed with the requisite amounts of liming materials. To each mixture enough distilled water was added to bring it to its optimum moisture content. The flasks were then weighed, stoppered with cotton plugs and stored in a dark room. Every few days the flasks were aerated, reweighed and water added to compensate for the loss due to evaporation. At intervals of 4, 6, 8 and 10 weeks, samples were withdrawn from the incubating flasks, air-dried and analysed for the phosphorus fractions by the method of Dean [1938] as modified by Ghani [1942]. The pH of the withdrawn samples was also determined at the same time. The treatment of the control was exactly the same except that no lime was added to it. The amounts of different materials added were calculated on the basis of top six inches of soil weighing 2,000,000 lb. per acre of land.

Each treatment was done in triplicate, but, as no appreciable variation could be observed in the results of the triplicate samples, the figures of single analysis only were included in the study. The changes in the fractions with time, effected by the different treatments, are shown separately in Tables I, II, III, IV and V. For convenience of comparing the effectiveness of different materials, the mean of the final increase or decrease over the control, as the case may be, in the three doses of dressings has been taken into account.

TABLE I

*Change in acetic acid-soluble phosphorus with time due to liming*  
(Mg.  $P_2O_5$  per 100 gm. of soil)

Treatment	Tons/ acre	4 weeks	6 weeks	8 weeks	10 weeks	Change in 10 weeks over control	Mean change in 10 weeks
Control . . .	..	2.7	2.6	2.7	2.6	..	..
$CaCO_3$ . . {	2.5	3.7	4.2	5.0	5.8	+3.2	+3.5
	5.0	3.9	4.6	5.2	5.9	+3.3	
	7.5	3.7	4.9	5.3	6.5	+3.9	
$Ca(OH)_2$ . {	2.5	3.7	5.2	6.0	6.7	+4.1	+4.0
	5.0	4.2	5.2	6.2	6.3	+3.7	
	7.5	4.1	5.6	6.3	6.8	+4.2	
$CaSO_4$ . . {	2.5	3.5	4.9	5.6	6.0	+3.4	+3.3
	5.0	3.6	4.9	5.4	5.6	+3.0	
	7.5	3.7	5.0	5.7	6.1	+3.5	
MgO . . . {	2.5	4.2	6.0	6.8	8.0	+5.4	+5.4
	5.0	4.3	5.9	7.0	7.8	+5.2	
	7.5	4.4	6.8	7.1	8.2	+5.6	

TABLE II  
*Change in organic phosphorus with time due to liming*  
 (Mg.  $P_2O_5$  per 100 gm. of soil)

Treatment	Tons/ acre	4 weeks	6 weeks	8 weeks	10 weeks	Change in 10 weeks over control	Mean change in 10 weeks
Control . . .	..	29.2	28.2	27.2	28.4	..	..
$CaCO_3$ . . . {	2.5	28.6	28.0	25.8	20.6	-7.8	-6.2
	5.0	27.6	25.0	26.8	21.8	-6.6	
	7.5	24.6	23.5	21.4	24.0	-4.4	
$Ca(OH)_2$ . . . {	2.5	25.8	23.4	23.0	23.8	-4.6	-6.4
	5.0	23.4	20.0	24.0	23.0	-5.4	
	7.5	21.4	20.4	18.8	19.2	-9.2	
$CaSO_4$ . . . {	2.5	25.8	25.0	26.4	23.2	-5.2	-5.1
	5.0	25.5	27.0	23.5	22.0	-6.4	
	7.5	25.6	25.0	21.5	24.8	-3.6	
MgO . . . {	2.5	24.0	22.0	22.0	16.2	-12.2	-11.1
	5.0	21.6	21.4	21.0	15.6	-12.8	
	7.5	21.4	23.0	21.0	20.0	-8.4	

TABLE III  
*Change in alkali-soluble inorganic phosphorus with time due to liming*  
 (Mg.  $P_2O_5$  per 100 gm. of soil)

Treatment	Tons/ acre	4 weeks	6 weeks	8 weeks	10 weeks	Change in 10 weeks over control	Mean change in 10 weeks
Control . . .	..	33.3	33.3	32.8	32.6	..	..
$CaCO_3$ . . . {	2.5	31.4	30.0	35.2	34.5	+1.9	+0.9
	5.0	31.4	31.4	34.2	34.2	+1.6	
	7.5	32.5	32.5	37.6	32.0	-0.6	
$Ca(OH)_2$ . . . {	2.5	33.6	34.1	32.0	31.2	-1.4	-1.9
	5.0	33.6	34.6	32.0	32.0	-0.6	
	7.5	34.6	34.6	32.0	28.8	-3.8	
$CaSO_4$ . . . {	2.5	32.5	32.0	33.6	32.8	+0.2	+0.7
	5.0	30.0	32.0	34.5	36.0	+3.4	
	7.5	31.4	32.0	34.5	31.2	-1.4	
MgO . . . {	2.5	34.1	34.1	28.0	28.8	-3.8	-3.8
	5.0	33.6	34.6	28.0	29.6	-3.0	
	7.5	33.6	33.0	28.0	28.0	-4.6	

TABLE IV

*Change in sulphuric acid soluble phosphorus with time due to liming*  
(Mg.  $P_2O_5$  per 100 gm. of soil)

Treatment	Tons/ acre	4 weeks	6 weeks	8 weeks	10 weeks	Change in 10 weeks over control	Mean change in 10 weeks
Control . . .	..	7.7	7.6	8.0	7.8	..	..
$CaCO_3$ . . .	2.5	7.8	7.2	8.2	7.6	-0.2	0.0
	5.0	7.8	7.4	8.2	7.8	0.0	
	7.5	7.8	7.4	8.2	8.0	+0.2	
$Ca(OH)_2$ . . .	2.5	7.6	8.2	8.8	8.6	+0.8	+0.8
	5.0	7.2	8.4	8.2	8.4	+0.6	
	7.5	8.0	8.0	9.0	8.8	+1.0	
$CaSO_4$ . . .	2.5	8.0	8.0	8.0	7.8	0.0	-0.1
	5.0	7.6	7.4	7.4	8.0	+0.2	
	7.5	7.8	7.6	7.8	8.2	-0.6	
MgO . . .	2.5	7.2	8.2	9.2	9.0	+1.2	+1.0
	5.0	7.8	8.2	9.0	9.2	+1.4	
	7.5	7.6	8.2	9.2	8.4	+0.6	

TABLE V

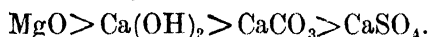
*Change in insoluble phosphorus with time due to liming*  
(Mg.  $P_2O_5$  per 100 gm. of soil)

Treatment	Tons/ acre	4 weeks	6 weeks	8 weeks	10 weeks	Change in 10 weeks over control	Mean change in 10 weeks
Control . . .	..	41.6	42.8	43.8	43.1	..	..
$CaCO_3$ . . .	2.5	43.0	45.1	40.3	46.0	+2.9	+2.5
	5.0	43.8	46.1	40.5	46.8	+3.7	
	7.5	45.8	46.2	42.2	44.0	+0.9	
$Ca(OH)_2$ . . .	2.5	43.8	43.6	44.7	43.2	+0.1	+1.8
	5.0	46.1	45.9	41.4	45.5	+2.4	
	7.5	46.4	45.9	48.6	46.1	+3.0	
$CaSO_4$ . . .	2.5	44.7	44.3	40.9	44.5	+1.4	+0.8
	5.0	47.8	43.2	45.6	42.2	-0.9	
	7.5	46.0	46.9	45.6	45.2	+2.1	
MgO . . .	2.5	45.6	44.2	48.5	52.3	+9.2	+8.4
	5.0	47.2	44.4	49.3	52.3	+9.2	
	7.5	47.5	43.5	50.0	49.9	+6.8	

## GENERAL DISCUSSION OF RESULTS

The data in the above tables show that the only fractions that are much affected by the treatments are the acetic acid-soluble (available) phosphorus and organic phosphorus. No appreciable change could be observed in the other two fractions, namely the alkali-soluble inorganic phosphorus (iron and aluminium phosphates) and phosphorus soluble in 2*N* sulphuric acid (apatites). The effect of varying quantities of the materials on the amount of changes produced is, however, very slight. The higher doses usually seem to be a little more effective.

It will be seen from Table I that acetic acid-soluble phosphorus regularly increases with time up to a period of 10 weeks for all doses of  $\text{CaCO}_3$ ,  $\text{Ca(OH)}_2$ ,  $\text{CaSO}_4$  and  $\text{MgO}$ . At the end of 10 weeks of incubation calcium carbonate has increased the fraction from the control value of 2.6 mg. to 6.1 mg., calcium hydroxide has increased it to 6.6 mg., calcium sulphate to 5.9 mg. and magnesium oxide to 8.0 mg. Magnesium oxide has trebled the quantity of available phosphorus, calcium hydroxide has increased it two and a half times while calcium carbonate and calcium sulphate have more than doubled it. It would thus appear that, of the four substances used, the effectiveness in increasing phosphate availability is of the order



There is not much difference in the behaviour of the last two substances and in fact their effect may be taken to be almost identical. The rate of increase (average of three dressings) of available phosphorus with time is shown graphically in Fig. 1.

Table II shows that organic phosphorus decreases with time for all doses of dressings with the four substances in question. In 10 weeks calcium carbonate has reduced the amount of organic phosphorus by 6.2 mg., calcium hydroxide by 6.4 mg., calcium sulphate by 5.1 mg. and magnesium oxide by 11.1 mg. As before, the highest change has been effected by magnesium oxide; it has reduced the fraction by more than one-third its original value. The data presented in Table II indicate that the order of effectiveness in breaking down organic phosphorus is



A comparison of Tables I and II will show that the decrease in organic phosphorus is not wholly accounted for by the corresponding increase in the available phosphorus. This is specially pronounced with magnesium oxide dressings. This would suggest that at least in the latter case a part of the phosphorus liberated by the decomposition of organic phosphorus combined in some other form which is not available. The diminution of organic phosphorus with time is shown graphically in Fig. 2.

It will also appear from Tables III and IV that alkali-soluble inorganic fraction (iron and aluminium phosphates) and sulphuric acid-soluble fraction (apatites) remain practically unchanged by the above treatments. The slight changes that have been produced in some cases are negligible in comparison with the amount of the fractions present in the soil. This would show that iron and aluminium phosphates do not contribute anything towards the increased availability caused by liming. The absence of any change in the

apatite fraction would also indicate that during the period of experiment no phosphorus has gone into apatite combination though calcium carbonate was present in excess in some of the treatments.

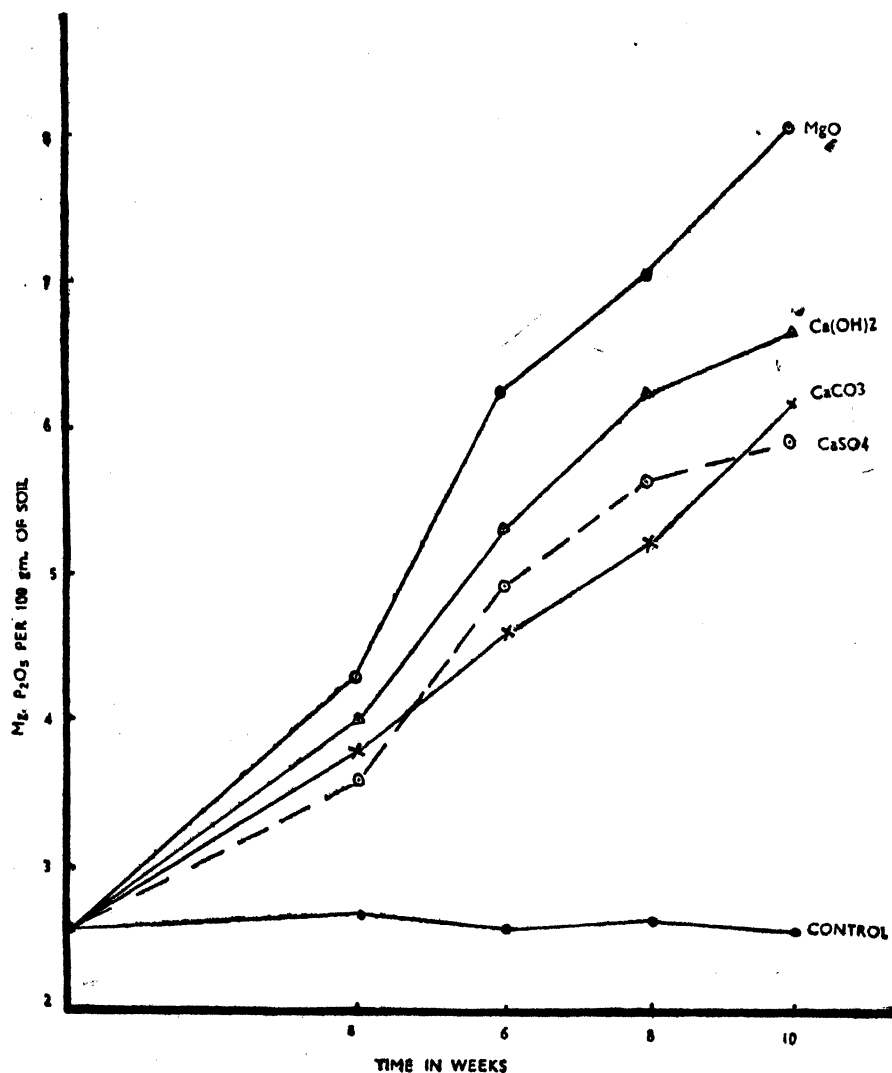


FIG. 1. Change in acetic acid-soluble phosphorus with time due to liming

The data in Table V represent the insoluble fraction, i.e. the difference between total phosphorus of the soil and the sum of the four phosphorus fractions determined. The treatments produced but little changes in this fraction except in the case of magnesium oxide. Magnesium oxide also caused a comparatively large decrease in organic phosphorus (Table II) all of which was not accounted for by the increase in the acetic acid-soluble fraction (Table I). In so far as it will permit a generalization it seems that with

magnesium oxide a part of the phosphorus liberated by the breakdown of organic phosphorus reverts to a highly inert combination. Dean [1938] found that long continued applications of superphosphate and sulphate of ammonia at Rothamsted and Woburn did not increase this fraction in any appreciable extent.

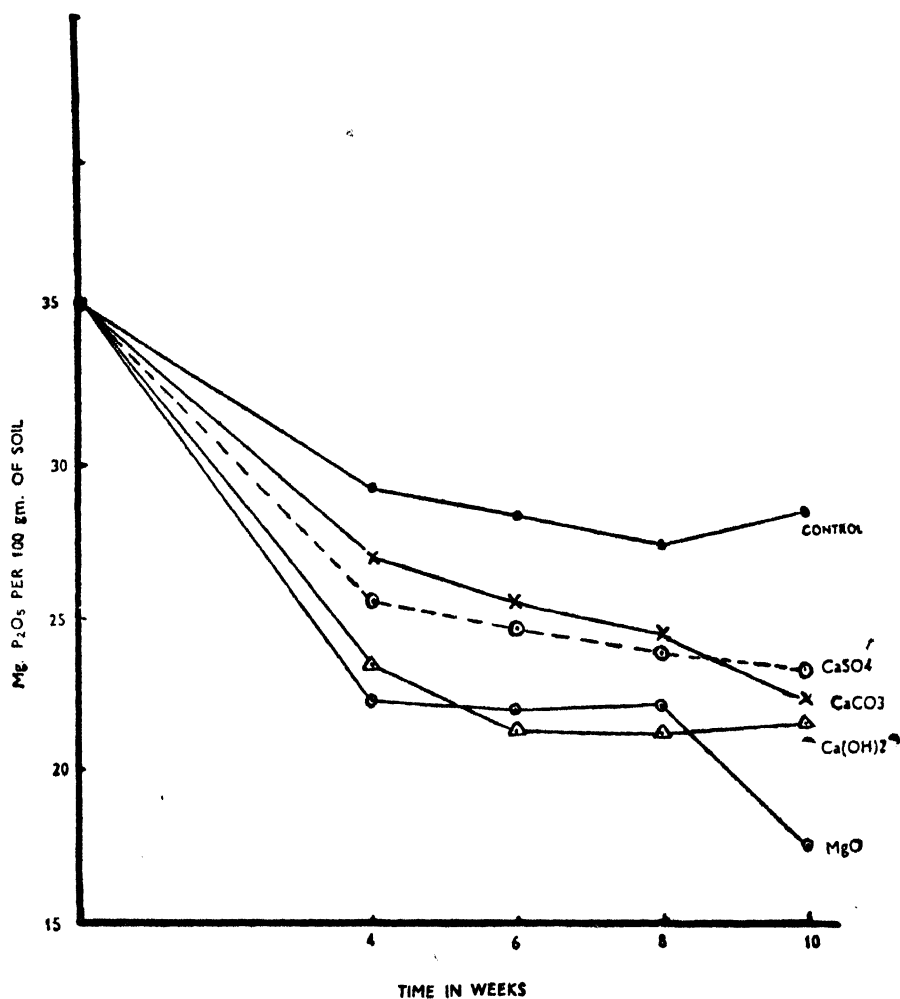


FIG. 2. Change in organic phosphorus with time due to liming

The change in the soil reaction with time brought about by the liming materials is shown in Table VI and Fig. 3. In all the treatments the *pH* of the media was increased, the higher doses showing slightly higher changes in all cases. In 10 weeks calcium carbonate shifted the *pH* from 4.7 to 7.2, calcium hydroxide to 7.9, calcium sulphate to 6.2 and magnesium oxide to 8.3. The order of effectiveness in increasing *pH* is, therefore,



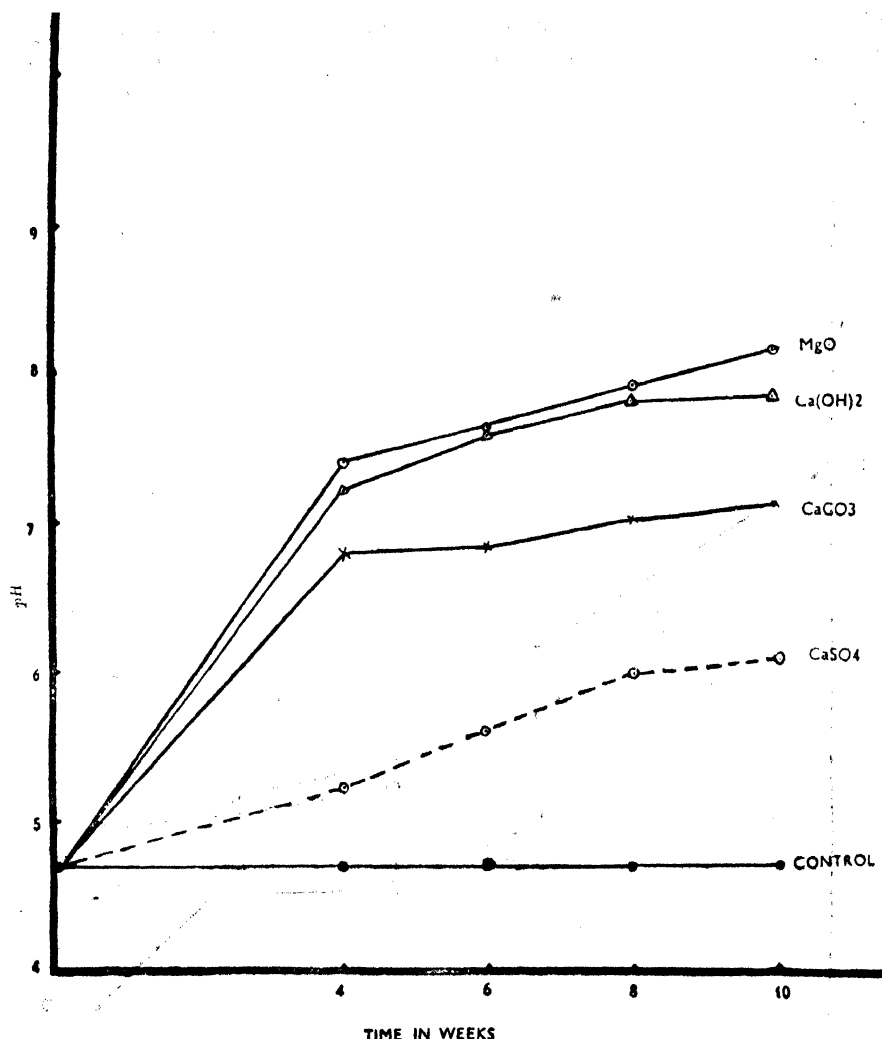


FIG. 3. Change in pH with time due to liming

Naftel [1937] using calcium carbonate at different calcium saturations has shown that one month after liming the pH was increased from 4.7 to 7.3 at 75 per cent calcium saturation. A mixture of calcium carbonate and magnesium carbonate gave similar results. The reaction of the soil was found to change linearly through the point of 75 per cent calcium saturation and then approached a maximum of approximately pH 8.0 at the equilibrium or saturation point.

It will also be seen that the materials stand in the same order in their effectiveness in increasing pH and available phosphorus and in decreasing organic phosphorus. It would thus be evident that the extent of upward shifting of the soil reaction is the principal factor in determining the degree of increased availability of soil phosphorus caused by liming. The change

in the soil reaction towards neutral conditions favours greater micro-biological activities which in turn effect greater breakdown of organic phosphorus compounds. The fact that any other fraction did not suffer any appreciable reduction by the treatments also shows that the increased availability may be ascribed solely to this cause.

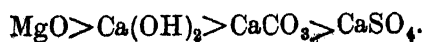
TABLE VI  
*Change in pH with time due to liming*

Treatment	Tons/ acre	4 weeks	6 weeks	8 weeks	10 weeks	Change in 10 weeks over control	Mean change in 16 weeks
Control . . .	..	4.7	4.7	4.7	4.7	..	..
CaCO <sub>3</sub> . . .	2.5	6.5	6.6	6.8	7.0	+2.3	+2.4
	5.0	6.8	6.8	7.0	7.2	+2.5	
	7.5	7.0	7.1	7.1	7.1	+2.4	
Ca(OH) <sub>2</sub> . . .	2.5	6.9	7.4	7.6	7.5	+2.8	+3.0
	5.0	7.2	7.6	7.8	7.7	+3.0	
	7.5	7.5	7.8	7.9	7.9	+3.2	
CaSO <sub>4</sub> . . .	2.5	5.0	5.3	5.9	6.1	+1.4	+1.4
	5.0	5.2	5.7	6.1	6.1	+1.4	
	7.5	5.3	5.9	6.1	6.2	+1.5	
MgO . . .	2.5	7.2	7.4	7.8	7.8	+3.1	+3.4
	5.0	7.4	7.6	7.8	8.2	+3.5	
	7.5	7.6	7.8	8.0	8.3	+3.6	

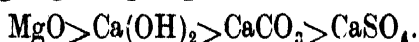
#### SUMMARY

An acid soil, having a very low amount of available phosphorus was incubated with calcium carbonate, calcium hydroxide, calcium sulphate and magnesium oxide, each at the rates of 2.5, 5 and 7.5 tons per acre. Transformation of soil phosphorus was studied by fractionating the samples at intervals of 4, 6, 8 and 10 weeks.

The available phosphorus regularly increased with time in all the treatments at all doses. The order of effectiveness in increasing phosphate availability was



The organic phosphorus decreased with time in all cases. The effectiveness in decomposing organic phosphorus was of the order



In all the treatments the *pH* of the soil was increased. The order of effectiveness in increasing *pH* was  $\text{MgO} > \text{Ca(OH)}_2 > \text{CaCO}_3 > \text{CaSO}_4$ .

The treatments did not produce any significant change in the other fractions.

The data reported show that the greater availability of soil phosphorus caused by liming, as observed here and by previous workers, is due to the decomposition of organic phosphorus compounds and not due to chemical interaction of the liming materials with the phosphates of iron and aluminium, as supposed by some.

#### ACKNOWLEDGEMENT

The authors wish to express their thanks to Prof. J. K. Choudhury, Ph.D. (Berlin), F.N.I., Head of the Department of Soil Science and Chemistry, for the keen interest he took in the work.

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NOTES ON THE INDIAN SPECIES OF SUGARCANE LEAF-  
HOPPER, *PYRILLA* STAL. (LOPHOPINAE :  
FULGOROIDAE

BY

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(With six text-figures)

INTRODUCTION

THE genus *Pyrilla* is widely distributed throughout the oriental region. The known species of this genus have been described by different workers. Owing to a great variability of the individuals of the same species great confusion usually occurs in identifying these leaf-hoppers. Over and above this, there is a general resemblance between the individuals of different species. This again causes a serious handicap for the field-worker to distinguish one species from the other.

The species of *Pyrilla* described by different workers are :—

- P. lycoides* Walker, *J. Ent.*, (i), [1862] ;
- P. aberrans* Kirby, *J. Linn. Soc. Zool.*, [1891] ;
- P. perpusilla* Walker, *List. Hom.*, (ii), [1851] ;
- P. pusana* Distant, *Ann. Mag. Nat. Hist.*, (8), 14, [1914] ;
- P. protuberans* Stal, *Ber. Ent. Zeitsch.*, 3, [1859] ;
- P. sumatrensis* Baker, *Treubia*, vol. 6, [1925.]

The Indian forms have been described by Distant in the *Fauna of British India—Homoptera*, vols. 3 and 6. He holds that three species of *Pyrilla*, viz. *P. aberrans*, *P. perpusilla* and *P. pusana* are distributed in various parts of the country. Later on Baker [1925] reviewed all the then known forms and described a new species from Sumatra (vide supra). The identification and validity of Indian species were questioned by a number of subsequent workers from different parts of India. Pruthi [1937] reviewed the problem of Indian species. He concludes that *P. aberrans* Kirby is unknown in India and that *P. perpusilla* and *P. pusana* are either synonyms or they are two varieties of one and the same species. He, however, retains these two species only due to the fact that the type specimen of *P. perpusilla* was not available to him for study.

The present writers took up the work in order to determine the species injuring sugarcane plants at Aligarh. Specimens were collected or acquired from various parts of the country. The collection thus raised included forms specially from Lyallpur (Punjab), Muzaffarnagar (United Provinces), Aligarh (United Provinces), Etah (United Provinces), Pusa (Bihar), Dacca (Bengal), Bhopal (Central India), Bombay, Coimbatore (Madras) and Ceylon.

The detailed morphological study of *Pyrilla* which is being conducted at the Aligarh Muslim University and is subsidized by the Imperial Council of Agricultural Research has proved of great avail in getting a clearer view of the characters on which different workers have based the distinction of different species.

Baker has based his classification of the species of *Pyrilla* on the contour of the rostrum and number of apical and sub-apical cells of the fore-wing. There is, however, a great variation and a regular gradation of these characters among the individuals of apparently the same species. With regard to the number of apical and sub-apical cells not only do the different sexes of the same species show a wide range of variability but even the right and left wings of the same individual differ in the number of these cells. It, therefore, often leads to a confusion while identifying one species from the other.

Baker's [1925] work is followed by that of Pruthi [1937]. Pruthi deals only with the male genitalia. This seems to be a limited view of the body structure of an entire organism and has apparently failed him to distinguish some of the well-defined species of the Indian sugarcane leaf-hoppers. The present work deals only with the description and identity of the Indian forms. The study of all the species occurring in the whole of oriental region is postponed for some later period when the present exigencies of war will be changed into more favourable conditions required for the work of this nature. The present writers avail of this opportunity to thank Dr H. S. Pruthi, Imperial Entomologist, for giving them access to the collection of *Pyrilla* present in the museum of the Imperial Agricultural Research Institute, New Delhi, and for providing opportunity of examining specimens identified by himself as well as by Distant. We acknowledge with gratitude the financial assistance of the Imperial Council of Agricultural Research and the help of the Entomologists-in-Charge of the above-mentioned sugarcane field stations in India and Ceylon for sending us the required material. Finally we are thankful to Dr M. B. Mirza, Chairman, Zoology Department, Muslim University, Aligarh, for providing all possible facilities and offering useful suggestions during the course of these studies.

The studies of the present writers have led them to the conclusions that in India two distinct species are found. They are *P. perpusilla* Walker, and *P. pusana* Distant. The specimens obtained from Ceylon are, however, quite different from those collected from any part of India. They are regarded by us as *P. aberrans* Kirby. A general description of these three species together with their distinctive features is given below:—

*Pyrilla perpusilla* Walker, (Pyrops) *List Hom.*, (ii), [1851] ;

*Fauna Br. India, Heteroptera-Homoptera*, vol. 3, [1906] ;

*Treubia*, vol. 6, [1925.]

The best specimens of this species were obtained from Bhopal and Bombay. It is, however, widely distributed all over India, chiefly at Lyallpur, Muzaffarnagar, Pusa, and in various Central and South Indian sugarcane tracts.

A typical male specimen has a uniformly ochraceous coloured body, slightly paler beneath than above. The body (Fig. 1-A) is much less robust than either *P. pusana* or *P. aberrans*. Fore-wings (Fig. 2-A) are semi-opaque,

more or less uniformly yellowish brown. Minute black spots are sparsely distributed all over the wing. The number of apical and sub-apical cells is highly variable and offers no sound characteristic of the species. Cephalic process (Fig. 1-a) is well developed. It is nearly two-fifth of the body length and is proportionally much longer than that of *P. pusana*. The dorsal margin of the cephalic process is generally parallel with that of the body (Fig. 1-A) and in a few cases slightly curved upwards at the tip. Male genitalia (Fig. 3-A) are very much smaller than those of either *P. pusana* or *P. aberrans* and are slightly different morphologically from either of them.

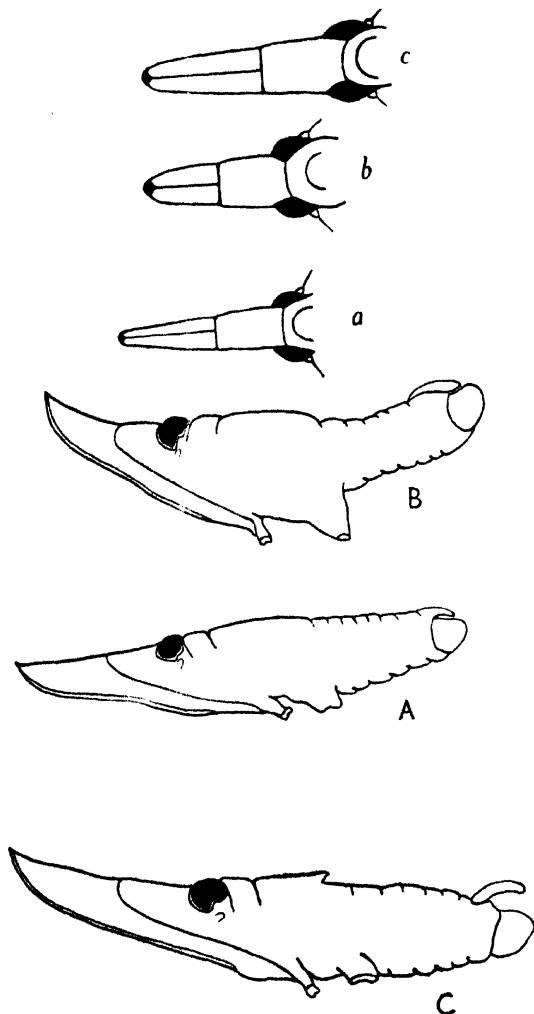


FIG. 1. Cephalic processes and body outlines of *P. perpusilla*, *P. pusana* and *P. aberrans*: a, b, c, cephalic processes; A, B & C, body outlines ( $\times 8$ )

Dorso-lateral margin of the ninth sternum (Fig. 4-A) which provides reliable morphological distinction between these three species is provided with a dome shaped elevation nearly in the middle. The tenth tergum (Fig. 5-A) which carries the anal tube is slightly concave and broadly truncated at the apex.

*Female.* The above description applies equally to the female. Female genitalia (Fig. 6-A) like those of the male are much smaller than those of *P. pusana* and *P. aberrans* and are also slightly different structurally.

*Pyrilla pusana* Distant. *Ann. Mag. Hist.*, (8), 14 [1914]; *Fauna B. India Rhynchota*, vol. 6, *Homoptera Appendix* [1916]. Good specimens of this species were obtained from Dacca (Bengal). This species as mentioned above is widely distributed in this country.

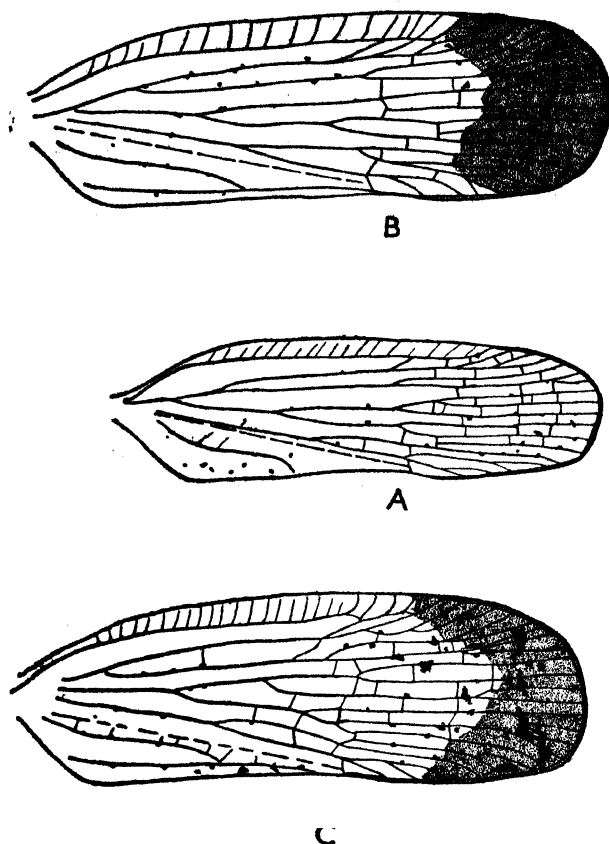


FIG. 2. Tegmina of *P. perpusilla* (A), *P. pusana* (B) and *P. aberrans* (C) ( $\times 8$ )

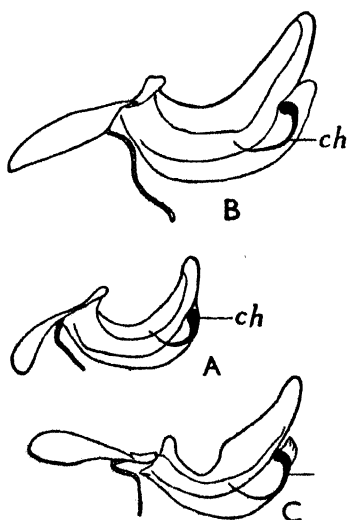


FIG. 3. Male genitalia of *P. perpusilla* (A), *P. pusana* (B) and *P. aberrans* (C) ( $\times 8$ )

In male, the body (Fig. 1-B) is much more robust and darkly coloured than that of *P. perpusilla*. Tegmina are dark ochraceous, the apical third (Fig. 2-B) being much darker in hue than the rest of the wing. Black spots are chiefly distributed in the apical region.

Cephalic process (Fig. 1-B) is comparatively much shorter than that of *P. perpusilla* or *P. aberrans*, strongly up curved towards the end. Its length is less than one-third of the total length of the body. The dorsal margin is not in line with that of the body but forms a saddle with it. Number of apical and sub-apical cells is very variable in different individuals as well as in different sexes. Tergum of the tenth segment (Fig. 5-B) is strongly

convex at the base and broadly truncated at the tip. Male genitalia (Fig. 3-B) are much larger than those of *P. perpusilla*. Conjectival hook of the phallus is very strongly developed and slightly twisted in the middle. Each of the dorso-lateral margins of the ninth sternum has a knob-like process, not in the middle like *P. perpusilla*, but at the end of the proximal one-third of it (Fig. 4-B).

*Female.* The female has approximately the same structure as the male. Female genitalia are larger in size than those of *P. perpusilla* and are slightly different in structure as well.

The correct identification of this species has been a matter of great confusion especially in India. Baker has expressed his doubts regarding the validity of this species. He had no specimen of this species for his study

and thus failed to distinguish it from *P. aberrans*. Pruthi's statement that *P. aberrans* is unknown in India is probably correct. In the present study no specimen of Indian species of *Pyrilla* was found to be *P. aberrans*. It is unfortunate that Distant has incorrectly identified some of the Indian forms as *P. aberrans*. A close study of such specimens in conjunction with form found in Ceylon will show a considerable morphological difference. The present writers, therefore, conclude that there are only two species of *Pyrilla* in India. One of them is *P. perpusilla* Walker, and the other is *P. pusana* Distant. A re-description of *P. aberrans* Kirby is given below, as Kirby's description is not adequate to enable field workers to distinguish it from a closely allied species *P. pusana*.

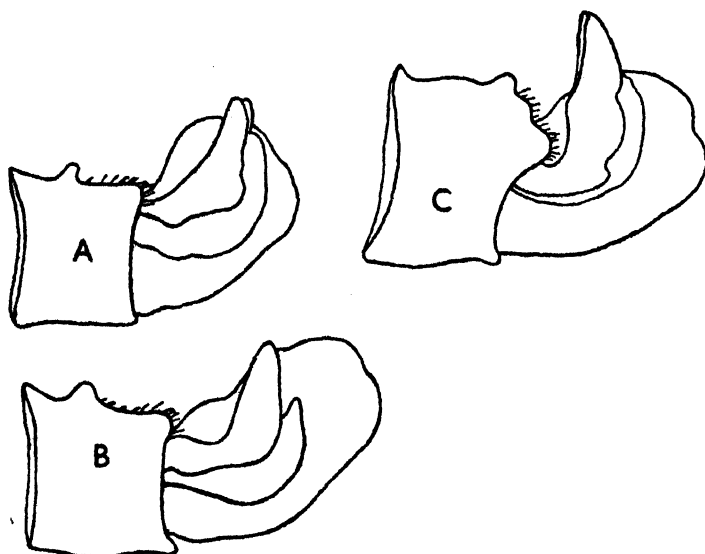


FIG. 4. Ninth segment with male genitalia of *P. perpusilla* (A), *P. pusana* (B) and *P. aberrans* (C) ( $\times 28$ )

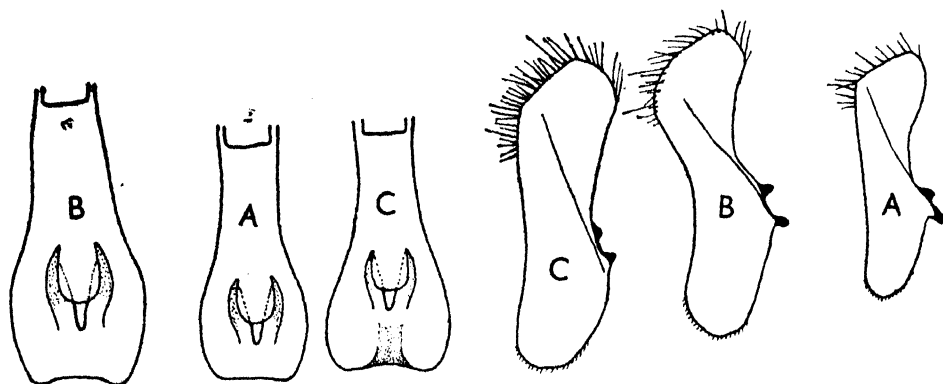


FIG. 5. Tenth tergum with anal tube of *P. perpusilla* (A), *P. pusana* (B) and *P. aberrans* (C) ( $\times 28$ )

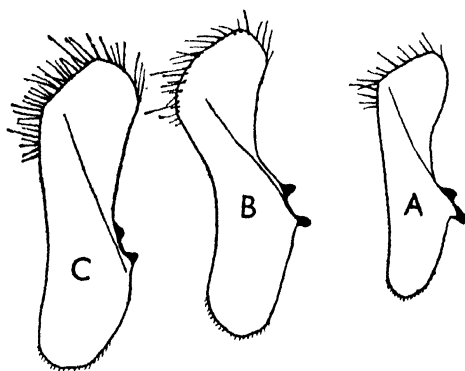


FIG. 6. Lateral valve of the ovipositor of *P. perpusilla* (A), *P. pusana* (B) and *P. aberrans* (C)

*Pyrilla aberrans* Kirby (Microchoria), *J. Linn. Soc. Zool.* 24, [1891] *Melich. Hom. Faun. Ceylon*, [1903]; (*Zamila aberrans*), *Fauna B. India Rhynchota*, vol. 3 [1906]; *Treubia*, vol. 6 [1925]. The specimens obtained from Ceylon were quite fresh and well preserved. In male, the body (Fig. 1-C) is quite robust, with yellowish-brown colour. Abdominal tergites are conspicuously reddish-brown. Fore-wings are ochraceous, the apical third being much darker than the rest. Black spots are chiefly distributed in the apical half and in many cases form two short transverse fuscous lines near the apical margin. Cephalic process (Fig. 1-C) is comparatively much longer than in *P. pusana*, nearly two-fifths as long as the entire body, and is curved upwards at the apex. The number of apical and sub-apical cells are variable in different individuals of the same sex as well as of different sexes. Tergum of the tenth segment (Fig. 5-C) is considerably different either from *P. perpusilla* or *P. pusana* and is deeply furrowed in the middle of its distal part. Male genitalia (Fig. 3-C) resemble those of *P. pusana* in broad outlines. The dorso-lateral margin of the ninth segment (Fig. 4-C), however, presents an important morphological distinction and appears to be a characteristic of this species. Unlike *P. pusana* and *P. perpusilla* it is divided into two parts, one antero-dorsal margin with three short elevations and the other postero-dorsal margin which is quite long and almost plain.

*Female.* The above description applies to the female as well, except or the characters particular to that sex.

#### SUMMARY

The above work contains a brief review of the studies on the Indian species of the sugarcane leaf-hopper *Pyrilla* Stal. It has been shown that in India two well-defined species are distributed at various places. They are *P. perpusilla* Walker and *P. pusana* Distant. A general description of the external morphology and various distinguishing features of the Indian species together with those of *P. aberrans* Kirby from Ceylon has been given with essential details.

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# DIFFERENTIATION OF HYDROGEN CLAYS AND HYDROGEN BENTONITES AND IDENTIFICA- TION OF MINERAL CONSTITUENTS CONTAINED IN THEM BY ELECTRO- CHEMICAL METHODS

## I. KAOLINITE AND KAOLINITIC CLAYS\*

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**T**HE hydrogen clay isolated from soil usually consists of one or more crystal-line secondary silicate minerals and varying quantities of 'free' oxides of Si, Al and Fe. The identification of the mineral constituents is of great interest and several physical methods, e.g. x-ray, optical and thermal analyses have been requisitioned for this purpose. Limitations of these methods are, however, known [Nagelschmidt, 1939]. They often do not go beyond indicating the group to which the mineral constituent of a given hydrogen clay belongs. Distinction between closely related individual members of the same group such as montmorillonite and beidellite is beset with considerable difficulties. Besides, all these methods throw very little light on their electro-chemical character which, after all, is most important as it determines the base exchange and many other chemical and physical properties of clays and soils.

The problem may be approached from an altogether different direction which does not appear to have been explored so far. It may be called the electro-chemical method of approach. The soil is essentially an electro-chemical or polar system and the central connecting theme in the electro-chemistry of soil is its dominant acid character. The hydrogen clay is the extreme acid form of the inorganic absorption complex obtained from soil. The electro-chemical properties of hydrogen clays isolated from several typical Indian soils have been discussed in previous publications [Mitra, 1936, 1940, 1942; Mukherjee

\*The results given in this paper have been taken from the published Annual Report for 1940-41 on the working of a scheme of research financed by the Imperial Council of Agricultural Research, India

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(J. N.), Mitra and Mukherjee (S), 1937 ; Mitra, Mukherjee (S) and Bagchi, 1940 ; Mukherjee (J. N.), Mitra, Chatterjee and Mukherjee (S. K.), 1942]. Valuable information for purposes of identifying the mineral constituents of a hydrogen clay and differentiating it may be obtained on comparing its electro-chemical properties with those of standard specimens of clay minerals. For sometime past studies of the more important of these minerals have been under way in this laboratory which have this object in view. This paper deals with the titration curves and other electro-chemical features of kaolinite and hydrogen clays prepared from the entire clay fraction of two lateritic soils which gave dehydration curves similar to those of kaolinitic minerals. Alterations in some properties of these hydrogen clays consequent on the removal of their free inorganic oxides by the method of Truog *et al.* [1936] have also been recorded. A separation of these free oxides is desirable and, as will be shown later, even necessary for the identification of the mineral constituents of the above hydrogen clays by the electro-chemical method.

TABLE I

*Particulars regarding soils, hydrogen clays and sample of kaolinite used*

Lab. No.	Description of soil or mineral	Silica/sesquioxide ratio of entire clay fraction	Reference No. of corresponding hydrogen clay or hydrogen kaolinite
MI	Kaolinite from Singbhum	1.99	H-kaolinite
22	Red lateritic soil (acidic) from Government Farm, Dacca (Bengal), collected at a depth of 0 to 6 in.	1.99	L ; L <sub>d</sub> *
33	Bhata red laterite soil from Bilaspur (Central Provinces) collected at a depth of 0 to 6 in.	1.88	N ; N <sub>d</sub> *

\*Prepared from the entire clay fractions after separation of free inorganic oxides by the method of Truog *et al.* [1936].

### EXPERIMENTAL

Details of procedure adopted for the preparation of the hydrogen clays, their chemical analysis and the electro-chemical measurements including electrometric titration and estimation of base exchange capacity have been described elsewhere [Mitra, 1936, 1940]. The dehydration curves were obtained by the method of Kelley *et al.* [1936]. The hydrogen kaolinite was obtained on repeatedly leaching with 0.02N HCl the entire clay fraction separated from a 2 per cent suspension of the powdered air-dried sample.

### RESULTS

#### A. Properties of hydrogen kaolinite

##### (a) Chemical composition and dehydration curve

Fusion analysis of the hydrogen kaolinite gives SiO<sub>2</sub>, 53.9 per cent ; Al<sub>2</sub>O<sub>3</sub>, 45.5 per cent ; and Fe<sub>2</sub>O<sub>3</sub>, 0.5 per cent. Al<sub>2</sub>O<sub>3</sub>, 2SiO<sub>2</sub>, requires SiO<sub>2</sub>,

54.55 per cent ; and  $\text{Al}_2\text{O}_3$ , 44.45 per cent. The dehydration curve of the kaolinite given in Fig. 1 has the same form as reported by Kelley *et al.* [1936].

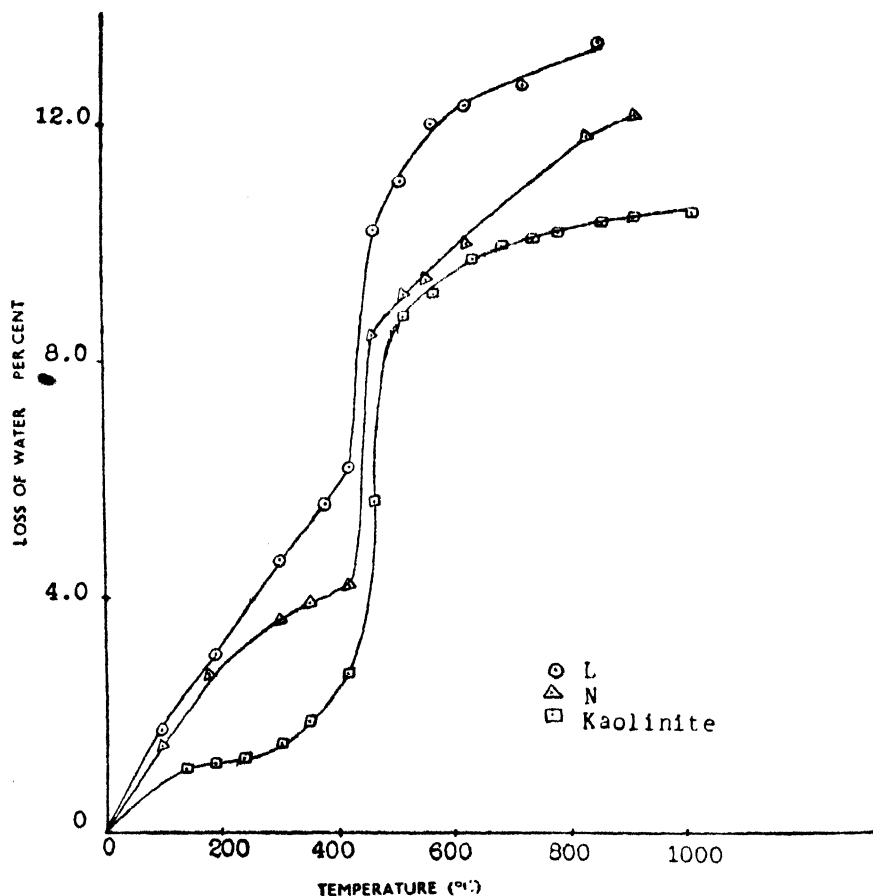


FIG. 1. Dehydration curves of hydrogen clays and hydrogen kaolinite

(b) *Existence of mobile  $H^+$  ions associated with the colloidal particles*

As previously observed with electro dialyzed silicic acid and hydrogen clay sols [Mukherjee, Mitra and Mukherjee, 1937 ; Mitra, 1936, 1940, 1942 ; Chatterjee, 1939] the colloidal particles of a stable sol of the hydrogen kaolinite carry with them mobile, i.e. osmotically active, hydrogen ions in electrical double layers surrounding the particles. This is evident on a comparison of the  $pH$  values of several hydrogen kaolinite sols with those of their ultrafiltrates recorded in Table II. The sol has a much lower  $pH$  than its ultrafiltrate. The difference between the two  $pH$ 's increases with the colloid content of the sol and illustrates the so-called suspension effect of Wiegner and Pallmann [1929].

TABLE II  
*pH values of hydrogen kaolinite sols and their ultrafiltrates*

Colloid content in gm. per litre	pH of sol	pH of ultrafiltrate
25.0	4.41	6.15
12.5	4.98	6.15
6.25	5.45	6.35
2.50	5.66	6.40

(c) *Osmotic and conductivity coefficients of the mobile H<sup>+</sup> ions*

In Table III the observed specific conductivities of the above hydrogen kaolinite sols have been compared with the values given by the expression

$$\frac{C_H^+ (U_H^+ + V_{\text{coll}})}{1000}$$

where  $C_H^+$  is the free acidity, and  $U_H^+$  and  $V_{\text{coll}}$  are respectively the mobilities of H<sup>+</sup> ion and the colloidal anion\*.

TABLE III  
*Observed and calculated specific conductivities of hydrogen kaolinite sols*

Colloid content of sol in gm. per litre	Sp. conductivity $\times 10^6$ mho	
	Observed**	Calculated
25.0	17.0	16.5
12.5	5.8	4.3
6.25	1.6	1.5
2.50	0.95	0.92

The observed and calculated values show satisfactory agreement. In the case of hydrogen clays and hydrogen bentonites, on the other hand, the observed values have been found to be much smaller than the calculated ones [Mittra, 1938, 1940 ; Hauser and Reed, 1937]. The difference is probably associated with the peculiarities of the structure of the various systems and the manner of distribution of the ions in the double layer associated with the colloidal particles.

\*For  $V_{\text{coll}}$  the value 20 has been taken

\*\*Corrected for the sp. conductivity of the ultrafiltrates

## (d) Features of titration curves with bases

Fig. 2 shows the titration curves, potentiometric and conductometric, of a 0.25 per cent suspension of the hydrogen kaolinite with dilute  $\text{Ba}(\text{OH})_2$ . Similar titration curves are obtained with  $\text{NaOH}$  and  $\text{Ca}(\text{OH})_2$ . The potentiometric curves with all three bases are shown in Fig. 3.

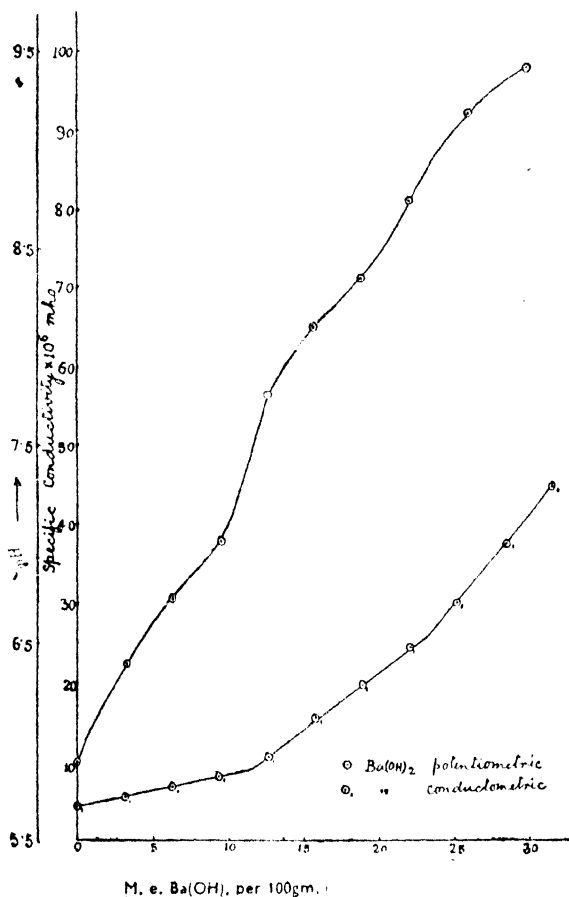


FIG. 2. Potentiometric and conductometric titration curves of hydrogen kaolinite with baryta

The potentiometric and conductometric curves (with all three bases) point to a weak dibasic acid character of the hydrogen kaolinite. No further inflexion point was observed on extending the titration (with 10N  $\text{NaOH}$ ) to pH 11.5.

The potentiometric titration curves with  $\text{NaOH}$ ,  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  though having the same form are not superimposable but have different slopes at any given pH (Fig. 3). The amount of the base required to reach a fixed pH is in the order  $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{NaOH}$ . The bases thus react with the sols in the same order. Similar observations have been previously made

with hydrogen clays and hydrogen bentonites [Mukherjee (J. N.), Mitra and Mukherjee (S.), 1937 ; Mitra, 1936, 1940, 1942 ; Mukherjee (J. N.), Mitra, Chatterjee and Mukherjee (S. K.), 1942]. The dependence of the reactivity of the base on the nature of its cation designated by Mukherjee, Mitra and Mukherjee [1937] as the irregular or specific cation effect is probably associated with the adsorbability of the cations in the dehydrated condition. The greater the adsorbability the larger is the quantity of  $H^+$  ions displaced from the double layer and neutralized at a fixed  $pH$ .

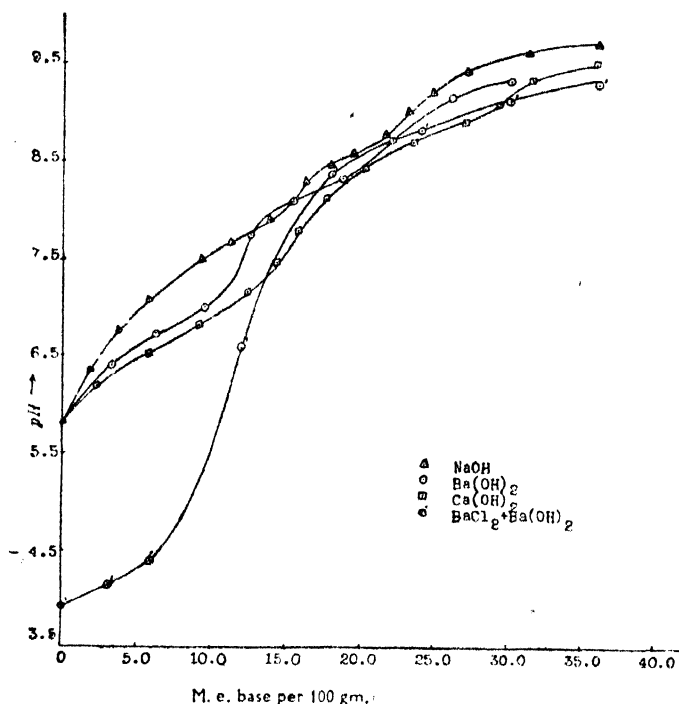


FIG. 3. Potentiometric titration curves of hydrogen kaolinite with different bases

Fig. 3 also gives the potentiometric curve obtained on titrating the clear supernatant liquid above the coagulum of a mixture of the hydrogen kaolinite and  $N BaCl_2$ . This curve, unlike that of the hydrogen kaolinite alone, has the appearance of that of a strong monobasic acid. This is expected as the supernatant liquid above the sol+salt mixture contains free  $H^+$  ions displaced from the double layer by the cations of the added  $BaCl_2$ . Features to be expected if  $Al^{+++}$  ions were present are not noticeable in the curve. This conclusion is supported by the analysis of the supernatant liquid which shows that aluminium is present in negligible quantities. The hydrogen kaolinite in this respect differs from hydrogen clays and hydrogen bentonites. Neutral salt extracts of the latter almost invariably contain appreciable quantities of  $Al^{+++}$  ions [Paver and Marshall, 1934 ; Mukherjee and Chatterjee, 1942 ; Chatterjee and Paul, 1942].

(e) *Degree of dissociation and dissociation constant of hydrogen kaolinite*

The degree of dissociation,  $\alpha$  (given by the ratio of the free acid to the total acid\* at the second inflexion point), of a 0.25 per cent suspension and the first and second dissociation constants  $K_1$  and  $K_2$  given by the *pH* values at 50 per cent neutralization referred respectively to the first and second inflexion points are recorded in Table IV. The last column of the table gives the second dissociation constant  $K_2^1$  calculated from the equation  $K_2^1 = \frac{c}{1-\alpha}$  where  $c$  is the total acid at the second inflexion point.

TABLE IV

*Degree of dissociation and dissociation constants of hydrogen kaolinite sols*

Base used	$\alpha \times 10^3$	$K_1 \times 10^7$	$K_2 \times 10^8$	$K_2^1 \times 10^8$
NaOH . . .	0.39	0.8*	2.4	2.9
Ba(OH) <sub>2</sub> . . .	0.41	2.3	7.4*	2.6
Ca(OH) <sub>2</sub> . . .	0.30	2.6	4.5	6.0*

$\alpha$  has a very small value. This is in agreement with the weak acid character of the titration curves of the hydrogen kaolinite and the low values of the dissociation constants recorded in Table IV. However, the same value of neither  $\alpha$  nor  $K_1$  or  $K_2$  is obtained from the titration curves with all three bases as would be expected in the case of a weak acid in true solution. The small value of  $K_1$  and  $K_2$  rather shows that the greater part of the  $H^+$  ions is present in a bound condition [Mukherjee, 1921, 1922] in electrical double layers surrounding the colloidal particles.

An approximate agreement between the different values of  $K_1$  as also of  $K_2$  is obtained if those marked with an asterisk (\*) in Table IV are neglected.  $K_1$  is then roughly ten times  $K_2$ .

(f) *Base exchange capacity of hydrogen kaolinite*

The base exchange capacities (b.e.c.) calculated from the titration curves are recorded in Table V.

TABLE V

*Base exchange capacity of hydrogen kaolinite calculated from titration curve*

Base used	B. e. c. in m. e. base per 100 gm. of oven-dried hydrogen kaolinite		
	At 1st inflexion point	At 2nd inflexion point	At <i>pH</i> 7.0
NaOH . . .	13.0(8.0)**	23.0(9.0)	5.0
Ba(OH) <sub>2</sub> . . .	12.0(7.55)	22.0(8.7)	9.0
Ca(OH) <sub>2</sub> . . .	15.5(7.70)	28.0(9.04)	11.0

\*It will be shown later that the total acid or the base exchange capacity calculated at the first or second inflexion point is not a fixed quantity but depends on the cation of the base used for the titration.

\*\* The figures in brackets denote the *pH* at the inflexion point.

The three bases do not give the same b. e. c. Calculated at a fixed pH, e.g. pH 7.0, it decreases in the order  $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{NaOH}$  in agreement with the irregular or specific cation effect. Calculated at the first or second inflexion point, however, the b. e. c. follows the order  $\text{Ca}(\text{OH})_2 > \text{NaOH} > \text{Ba}(\text{OH})_2$ . The smaller relative effect of  $\text{Ba}(\text{OH})_2$  compared with  $\text{NaOH}$  is really due to the lower pH at the inflexion point in the titration curve with  $\text{Ba}(\text{OH})_2$  than with  $\text{NaOH}$ . The pH effect thus masks the cation effect [Mukherjee, Mitra, Mukherjee and Chatterjee, 1942].

The ratio of the b. e. c.'s at the second and first inflexion points in the titration curves with all three bases is very nearly—it is actually slightly less than—2 as would be expected in the case of a dissolved dibasic acid.

The base exchange capacities estimated by Parker's and Schofield's methods [Parker, 1929 ; Schofield, 1933] as also by titration with  $\text{Ba}(\text{OH})_2$  in the presence of  $N \text{ BaCl}_2$  are given in Table VI.

TABLE VI

*Base exchange capacity of hydrogen kaolinite estimated by different method*

Method	B. e. c. in m. e. per 100 gm.
(i) Parker . . . . .	12.5(7.0)*
(ii) Schofield . . . . .	10.6(7.1)
(iii) Titration with $\text{Ba}(\text{OH})_2$ in the presence of $N \text{ BaCl}_2$	15.0(7.0)

\*The figures in brackets denote the pH at which the b. e. c. has been estimated

The b. e. c.'s obtained by the three methods are in the order (iii) > (i) > (ii). All three methods give values which are near about the b. e. c. at the first inflexion point in the titration curves with bases (in the absence of salts) but are much smaller than that given by the second inflexion point (Table V). The second inflexion point, therefore, indicates the neutralization of hydrogen ions which are present at a very high level of affinity and cannot be displaced from the double layer by such strongly adsorbed cations as  $\text{Ba}^{++}$  and  $\text{Ca}^{++}$  even when added in such high concentration as  $1N$  in methods (i) and (iii), and  $0.05N$  in method (ii). It appears that the pH effect is more potent than the cation effect in the estimation of these 'high affinity' hydrogen ions. In the above three methods, the b. e. c. is estimated at a much lower pH than that at which the second inflexion in the titration curves is observed.

B. *Properties of hydrogen clays giving dehydration curves similar to that of kaolinitic minerals*

The chemical compositions and the base exchange capacities calculated from titration curves are given in Tables VII and VIII. The titration curves of L are shown in Fig. 4. N gives similar titration curves and these have been omitted. The titration curves of  $L_d$  and  $N_d$  (obtained from L and N after separation of the free oxides by the method of Truog *et al.* [1936]) are given in Figs. 5 and 6. The dehydration curves are shown in Fig. 1.

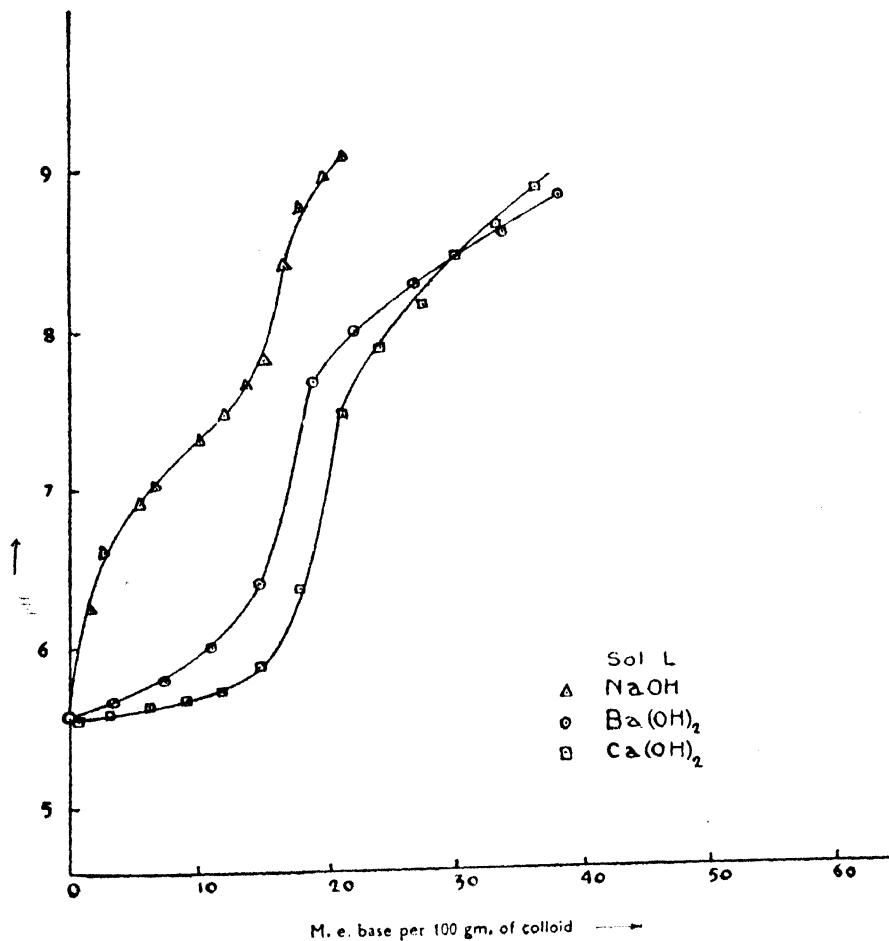


FIG. 4. Potentiometric titration curves of hydrogen clay L with different bases

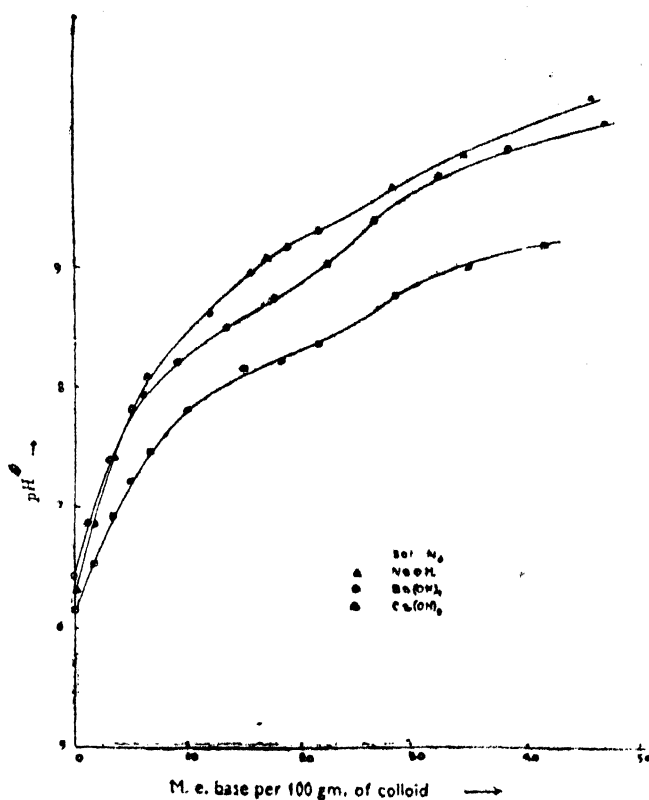


FIG. 5. Potentiometric titration curves of hydrogen clay  $L_d$  with different bases

TABLE VII

*Chemical compositions of hydrogen clays before and after separation of their free inorganic oxides*

Reference number of hydrogen clay	Chemical composition on the ignited basis		
	$\text{SiO}_2$ (per cent)	$\text{Al}_2\text{O}_3$ (per cent)	$\text{Fe}_2\text{O}_3$ (per cent)
$L$	51.2	36.0	12.0
$L_d$	57.5	38.0	5.7
$N$	42.6	3.7	54.2
$N_d$	61.0	34.3	4.6

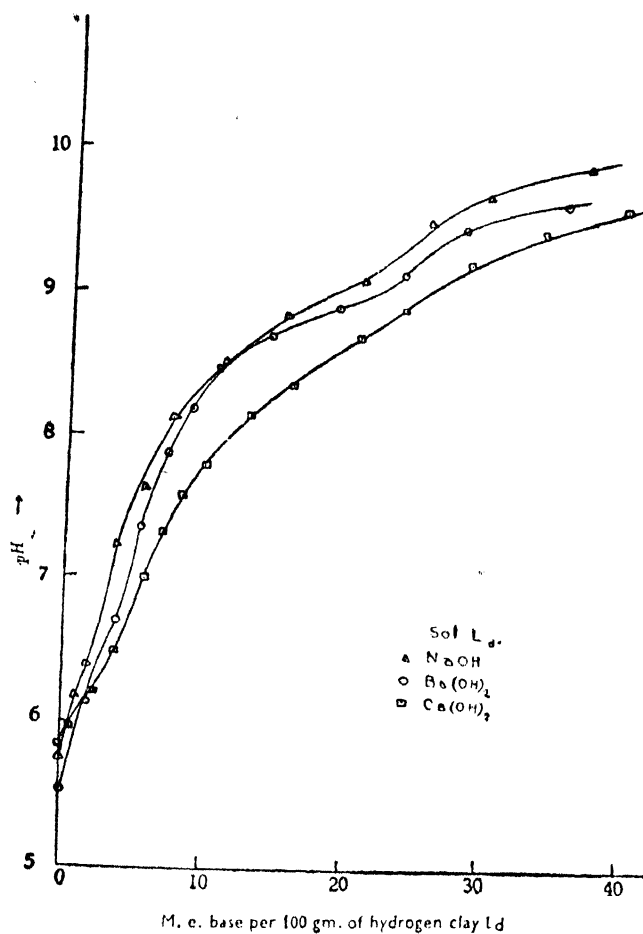
FIG. 6. Potentiometric titration curves of hydrogen clay  $\text{Na}$  with different bases

TABLE VIII

*Base exchange capacity of hydrogen clays before and after separation of their free inorganic oxides*

Reference number of hydrogen clay	B. e. c. in m. e. per 100 gm. of hydrogen clay at inflexion point of titration curve with		
	NaOH	$\text{Ba}(\text{OH})_2$	$\text{Ca}(\text{OH})_2$
L	16.3(8.2)*	17.5(7.1)	19.0(6.8)
$\text{L}_d$	4.0(7.1) ; 24.5(9.5)	5.0(7.16) ; 23.5(9.0)	5.5(7.0) ; 25.0(9.06)
N	18.8(7.5)	19.0(7.0)	20.5(6.5)
$\text{N}_d$	26.5(9.5)	28.4(9.5)	27.0(8.6)

\*The figures in brackets denote the pH at the inflexion point of the titration curve

The dehydration curves of L and N (Fig. 1) have features common with those of the kaolinite\*. The adsorbed water forms a comparatively small percentage of the total water and the inflexion point lies near about 400°C. The titration curves, however, present quite dissimilar features. Thus the dibasic acid character observed with the hydrogen kaolinite (Figs. 2 and 3) is not shown by the titration curves of L (Fig. 4) and N. Moreover, while the NaOH curve of these two hydrogen clays resemble that of a weak monobasic acid, their  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  curves reveal a strong or moderately strong acid character. These features have been observed with the majority of the hydrogen clays studied by us [Mitra, 1940, 1942; Mukherjee, Mitra, Chatterjee and Mukherjee, 1942].

Unlike L and N, the derivatives  $\text{L}_d$  and  $\text{N}_d$  behave as a weak acid judged from the form of their titration curves with all three bases (Figs. 5 and 6). Like the hydrogen kaolinite, the titration curves of  $\text{L}_d$  have the appearance of that of a weak dibasic acid. The base exchange capacity at the second inflexion point has nearly the same value (about 25 m.e. per 100 gm.) and occurs at approximately the same pH (near about 9.0). The base exchange capacity at the first inflexion point of  $\text{L}_d$ , however, has a definitely lower value than the hydrogen kaolinite and it occurs at a lower pH.

The chemical composition of  $\text{L}_d$  approaches that of the hydrogen kaolinite. The chemical, electro-chemical and dehydration data thus all lead to the conclusion that kaolinite is the dominant mineral constituent of the clay fraction of the Dacca lateritic soil. Free inorganic oxides contained in L probably mask the electro-chemical features characteristic of kaolinite. These features are, however, observed in the derivative  $\text{L}_d$ .

Unlike  $\text{L}_d$ ,  $\text{N}_d$  does not behave as a dibasic acid. Its titration curves all reveal a weak monobasic acid character. Their inflexion point, however, occurs in the same range of pH (8.5 to 9.5) as the second inflexion in the titration curves of  $\text{L}_d$  and the hydrogen kaolinite. The base exchange capacity of  $\text{N}_d$  calculated from the only inflexion point in its titration curve has approximately the same value (near about 25 m. e. per 100 gm.) as the base exchange capacities of  $\text{L}_d$  and the hydrogen kaolinite calculated from the second inflexion point.

The chemical composition of  $\text{N}_d$  is also materially different from that of kaolinite. The chemical and electro-chemical evidences obtained with N and  $\text{N}_d$  can be reconciled with the dehydration data if it is assumed that (a) N contains kaolinite or more probably some other mineral of the kaolin group mixed with some free oxides which have not been completely removed as a result of the treatment given for this purpose and which are, therefore, not altogether absent in  $\text{N}_d$  and/or that (b) in addition to a large percentage of a kaolinitic mineral N and  $\text{N}_d$  contain one or more secondary clay minerals belonging to a different group.

### SUMMARY

Marked acidic properties are shown by a hydrogen kaolinite prepared from the entire clay fraction of a sample of kaolinite from Singbhum (Bengal)

\*That the curves are not exactly similar may be due to several factors, e.g. large differences in the average size of the particles [Kelley, *et al.* 1936], presence of small quantities of minerals other than those of the kaolin group, etc.

by repeatedly leaching it with dilute hydrochloric acid. Its potentiometric and conductometric titration curves with  $\text{NaOH}$ ,  $\text{Ba}(\text{OH})_2$  and  $\text{Ca}(\text{OH})_2$  all reveal a weak dibasic acid character. The first and second dissociation constants are of the order of  $10^7$  and  $10^8$  respectively. The ratio of the base exchange capacities raise at the second and first inflexion points is very nearly 2.0 and the base exchange capacity at the second inflexion point is about 25 m.e. per gm.

The hydrogen clay prepared from a red lateritic soil from Dacca which gives a dehydration curve similar to that of kaolinitic minerals also shows a weak dibasic acid character after separation of its free inorganic oxides by the method of Truog *et al.* and the base exchange capacity at the second inflexion point is near about 25 m.e. per 100 gm. If the free oxides are not removed this hydrogen clay behaves as a monobasic acid judged from the nature of the titration curves. The dibasic acid character is not observed on titrating another hydrogen clay prepared from a Bhata red laterite soil either before or after removal of its free inorganic oxides by the above method, though this hydrogen clay also gives a dehydration curve similar to that of kaolinitic minerals.

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# SELECTED ARTICLE

## GENE SYMBOLS FOR USE IN COTTON GENETICS\*

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**G**ENETIC work on cotton has reached a stage where the haphazard allocation of gene symbols adopted in the past has become inconvenient and confusing. We have listed the cotton genes of which descriptions are known to us and attempted to adjust their nomenclature in accordance with accepted genetic conventions and with regard to the special circumstances obtaining in the genus *Gossypium*.

The conventions we have adopted are as follows :

- (1) Multiple allelomorph series : A series of alphabetic superscripts to a common gene symbol, e.g. the leaf shape series,  $L^B$ ,  $L^I$ ,  $L^L$ ,  $L$ ,  $l$ .
- (2) Duplicate factors : The same gene symbol with numerical subscripts, e.g. the chlorophyll deficient duplicates,  $Chl_1$ ,  $chl_1$  :  $Chl_2$ ,  $chl_2$ .
- (3) Complementary factors : The same gene symbol with alphabetic subscripts, e.g. complementary crumpled,  $Cp_a$ ,  $cp_a$  :  $Cp_b$ ,  $cp_b$ .

A special difficulty arises in the nomenclature of cotton genes. Many characters occur in both Old World and New World groups, and frequently in the wild species also. Their genetic basis is usually similar, and in one case it has been shown that the controlling genes are homologous<sup>9</sup>. It is, therefore, desirable to have a common gene terminology throughout, but the high degree of sterility in crosses between groups makes the demonstration of homology a slow and difficult undertaking. We propose, therefore, that the nomenclature of the Old World ( $2n=26$ ) cottons be taken as the basis, and genes controlling homologous characters in the New World cultivated and Polynesian wild ( $2n=52$ ) and New World wild ( $2n=26$ ) species will be given the same symbols, but printed in ordinary type until homology has been proved, and then in italics.

Our proposals for the anthocyanin multiple allelomorph system require special explanation. The original series established in Asiatic cottons<sup>10</sup> consisted of six members. The two lowest members were spotless, and the four higher members, in addition to giving red petal spot, determined progressive extension of vegetative anthocyanin expression. The six members were arranged serially,  $R$ ,  $R^L$ ,  $R^C$ ,  $R^S$ ,  $rg$ ,  $r^o$ . Recently, however, a spotless equivalent

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TABLE I

*List of recorded genes in cotton and their present and proposed symbols*  
(See text for explanation of nomenclature)

Character	Old World (2n = 26) cottons			New World (2n = 52) cottons		
	Gene effect	Present symbol and authority	Proposed symbol	Gene effect	Present symbol and authority	Proposed symbol
Chlorophyll deficiency	green-chlorophyll deficient	(43) <sup>b</sup>	(41)	green-chlorophyll deficient duplicate	chl <sup>a</sup> (11, 29) chl <sup>b</sup>	Ch <sub>1</sub> Ch <sub>2</sub>
	green-virescent yellow	V <sub>1</sub> (45)	<i>Ne alter.</i>	green + virescent yellow	V (26)	v
Crumpling	crumpled (majority)	A (16)	C <sub>ph</sub> C <sub>ph</sub>			
Crinkled dwarf				normal-crinkled	Cr (5)	Cr
Cluster habit				normal-cluster	C <sup>1</sup> (40)	C <sub>1</sub>
Short fruiting branches				long-short sympodia	S <sup>h</sup> (24)	Sb
Curly leaf	curly	C <sub>u</sub> (45)	<i>No alteration</i>			
Leaf shape	mutant broad	L <sup>L</sup> (18)	<i>No alteration</i>	super-okra okra normal	O <sup>s</sup> (10, 57) O <sup>o</sup> O <sup>n</sup>	L <sup>s</sup> L <sup>o</sup> l
	mutant intermediate	L <sup>1</sup>				
	laciniated	L <sup>L</sup>				
	narrow	L				
	broad	l				
Leaf nectaries	present - absent	(27)	<i>Ne</i>			
Anthocyanin	Spotted series			Spotted series		
	red plant body	R (17)	R <sub>1</sub> <sup>sp</sup>	red leaf	R <sup>2, 3, 4, 5, 6</sup> (6, 9, 10, 23)†	R <sub>1</sub> <sup>sp</sup> (12)
	red leaf	R <sup>L</sup>	R <sub>1</sub> <sup>sp</sup>			
	red calyx	R <sup>C</sup>	R <sub>1</sub> <sup>sp</sup>	tinged stem	S <sup>1, 2, 3</sup> (8)	R <sub>1</sub> <sup>sp</sup> at 47
	tinged stem	R <sup>S</sup>	R <sub>1</sub> <sup>sp</sup>			
	green stem	r <sup>S</sup>	R <sub>1</sub> <sup>sp</sup>			
	Spotless series			Spotless series		
	red leaf	R <sub>2</sub> <sup>0</sup> (22)	R <sub>1</sub> <sup>sp</sup>	tinged stem	s <sup>0</sup> (6)	R <sub>1</sub> <sup>sp</sup>
	tinged stem	r <sup>0</sup>	R <sub>1</sub> <sup>sp</sup>			
	Duplicate (ex. <i>G. anomalum</i> )		R <sub>1</sub> <sup>sp</sup> (11)	Duplicate red spotless	R <sup>1</sup> (3, 10, 26, 40, 42, 44)	R <sub>1</sub> <sup>sp</sup>
	Spot reducer		Sr (22)			
Corolla color	yellow petal	Y (15)	Y <sub>1</sub>	yellow	Y <sup>h</sup> (8, 14)	Y <sub>1</sub>
	pale	Y <sup>p</sup>	Y <sub>1</sub> <sup>+</sup>	cream	y	Y <sub>1</sub> <sup>+</sup> 1
	white	y	y <sub>1</sub>			
	pale, complementary		Y <sub>1</sub> <sup>+</sup> (24)			
	pale, complementary (ex. <i>G. anomalum</i> )		1 <sub>1</sub> <sup>+</sup> (25)			
	yellow depressor		Yd <sub>1</sub> (10)	yellow duplicate	Y <sup>D</sup> (14)	Y <sub>2</sub>
Pollen color	yellow		P, P <sub>1</sub>	yellow	P (7)	P
	pale } complementary	(29)	P <sub>1</sub> (38)			
	cream }		P <sub>1</sub> (38)	cream	p	p
Moristic variant	increase in number of floral parts - normal	(30)	M			

\* In this paper the authors refer to two types of chlorophyll deficiency, only one of which behave as a simple recessive. To the recessive we assign the symbol chl<sup>a</sup>.

† Red leaf mean spot of Trinidad Red Leaf and red leaf extinguished spot of Cassava have both been transferred to the Sea Island background, on which they are indistinguishable. R<sup>2, 3, 4, 5, 6</sup> and R<sup>2, 3, 4, 5, 6</sup> are therefore identical and may be given the symbol R<sub>1</sub><sup>sp</sup>.

TABLE I—*contd.*

Character	Old World (2n = 26) cottons			New World (2n = 52) cottons		
	Gene effect	Present symbol and authority	Proposed symbol	Gene effect	Present symbol and authority	Proposed symbol
Sterility	fertile - sterile	(20)	<i>Stp</i>			
Female sterility	fertile - female sterile	<i>Stg</i> (41)	<i>No attraction</i>			
Petaloidy	normal - petaloidic	<i>pPd</i> (31)	<i>Pdy</i>			
Gill dehiscence	more dehiscence - less dehiscence	(1)	<i>De</i>			
Lint colour	khaki - white	<i>K</i> (16)	<i>lc<sub>1</sub><sup>a</sup>, lc<sub>2</sub></i>	khaki - white	<i>lc<sub>1</sub><sup>H</sup></i> (13)	<i>Lo<sub>1</sub><sup>a</sup> - lo<sub>1</sub></i>
	khaki, duplicate -		<i>lc<sub>2</sub><sup>a</sup></i> (71)	duplicate	<i>lc<sub>2</sub><sup>H</sup></i> (13)	<i>Lo<sub>2</sub><sup>a</sup> - lo<sub>2</sub></i>
	light brown	<i>D<sub>1</sub></i> (19)	<i>lc<sub>3</sub><sup>a</sup></i> (31)			
	white		<i>lc<sub>4</sub></i>			
	light brown-white, duplicate	<i>D<sub>2</sub></i> (19)	<i>lc<sub>5</sub><sup>a</sup>, lc<sub>6</sub></i> (73)	green - white	<i>ol</i> (9)	<i>Lg</i>
Lintlessness	hairy linted-glabrous lintless (complementary)	<i>H<sup>0</sup></i> (2)	<i>ll<sub>1</sub>, ll<sub>2</sub></i> (21)			
	hairy linted - hairy lintless (complementary)		<i>ll<sub>1</sub>, ll<sub>2</sub></i> (21)			
	hairy linted - hairy lintless (sometimes lethal)	<i>h<sup>0</sup>ll<sup>1</sup></i> (2)	<i>ll<sub>1</sub> - ll<sub>2</sub></i> (21)*			
Seed fuzz				naked (low lint index)-fuzzy (Upland)	<i>N,L</i> (3,4,23, 40,43)	<i>Pn</i>
	tufted - fuzzy	<i>T</i> (19)	<i>F<sub>1</sub></i>	tufted - naked (Peruvian)	<i>T</i> (3,10)	<i>Pt</i>
				less fuzzy - more fuzzy (Peruvian)	<i>S<sup>0</sup></i> (25, 10)	<i>Pn</i>
				tufted - fuzzy (Upland)	<i>S<sup>1</sup></i> (3)	<i>Pn</i>
Seed fuzz colors				green - white	<i>G</i> (3)	<i>Fg</i>
				brown - white	<i>B<sup>1</sup></i> (3)	<i>Fbr</i>

\* Afzal & Hutchinson (2) assigned the capital letter (*H<sup>1</sup>*) to the "dominant" lethal. Hutchinson & Gidhari (21) showed that the heterozygote was strictly intermediate and the homozygote sometimes viable. On account of the close parallelism between Punjab

of *R<sup>1</sup>* has been described by Hutchinson and Ghose<sup>22</sup> and was given the symbol *R<sub>2</sub><sup>0</sup>*. While this is satisfactory for genes now recognized, it seems to be desirable to devise a system of nomenclature which will cover any spotless types which may be found in the future. From the fact that the new spotless type is, like *ro*, complementary with *rk* with reference to petal spot<sup>17,22</sup> it appears that there are probably at least two independent gene centres on the *R* protosome<sup>18</sup>, affecting respectively petal spot and presence and distribution of anthocyanin. The probable relationship between the spotted and spotless series is shown below, where the proposed symbols are indicated. The present ones are in brackets.

	Petal spotted	Spotless
Red plant body	<i>R<sub>1</sub><sup>25</sup></i> ( <i>R</i> )	
Red leaf	<i>R<sub>1</sub><sup>18</sup></i> ( <i>R<sub>1</sub></i> )	<i>R<sub>2</sub><sup>10</sup></i> ( <i>R<sub>2</sub><sup>0</sup></i> )

	Petal spotted	Spotless
Red calyx	$R_2^{CS}$ ( $R^C$ )	
Red tinged stem (i.e. basic anthocyanin gene present, expression variable but slight)	$R_2^{AS}$ ( $R^S$ )	$R_2^{AO}$ ( $r^0$ )
Green stem, ghost spot (basic anthocyanin gene absent)	$R_2^{AS}$ ( $r^G$ )	

Such symbolisation has also been attempted for the New World cottons on the basis of phenotypic appearance. The fact that members of the New World  $R_2$  series are printed in italics indicates only that homology of the locus with that of Asiatic cottons has been established. In no case is it intended to imply identity of allelomorphs.

In Table I are listed the characters that have been studied genetically, the gene symbols allotted to them, and the alterations we propose. The numbers in brackets refer to the list of reference attached at the end.

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